Biodiv. Res. Conserv. 3-4: 251-257, 2006

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Distribution and comparison of two morphological forms of water soldier (*Stratiotes aloides* L.): a case study on Lake Słosineckie Wielkie (Northwest Poland)

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Abstract: The investigation was aimed at demonstrating differences between the floating forms of *Stratiotes aloides* L. and the submerged one. The following plant features of 40 specimens and 909 leaves were examined: width, length, sex, number of generative and vegetative organs, dry mass of whole plants and their organs, leaf apex angle, leaf width measured 3 cm from the base, 10 cm from the base and 10 cm from the top, leaf area, cellular wall thickness, number of vascular bundles and number of chloroplasts. The leaf area was determined with an image analyzer Met-Ilo 8. Leaf cells were examined with the use of a confocal and optical microscope. Water and bottom deposits from the places of specimens collection were analysed. The results confirm the occurrence of two morphological forms of *S. aloides* in Lake Słosineckie Wielkie as well as the morphological and anatomical differences between them.

Key words: *Stratiotes*, water soldier, variability, morphological forms, taxonomy

1. Introduction

Stratiotes aloides L. is a perennial of the Hydrocharitaceae family, widely distributed in Europe (Cook & Urmi-König 1983). In Poland *S. aloides* occurs in the waters of the whole lowland area (Szafer *et al.* 1986; Podbielkowski & Tomaszewicz 1996). It is a dioecious plant often forming unisexual populations, although in some populations both female and male individuals have been observed. It has been stated that in Poland *S. aloides* can flower and bear fruit (Mądalski 1938; Toma 2001).

Previous studies on the species dealt with, among others, fruiting and seeds sprouting (Smolders *et al.* 1995a, 1995b), development dynamics, turions, offsets, morphological forms and phenology (Glück 1936; Kornatowski 1976, 1979, 1983/84, 1985) as well as life cycle (Renman 1989) and ecology (Strzałek 2004). The revision of the whole genus was made by Cook & Urmi-König (1983). However, there is a significant gap in the studies on *S. aloides* concerning the morphological and anatomical differences between its various forms. In regard to this the following research goals have been established: (*i*) ascertainment which forms of *S. aloides* occur in Lake Słosineckie Wielkie; (*ii*) determination of their distribution in the lake; (*iii*) demonstration of the morphological and anatomical differences between various forms of *S. aloides*; (*iv*) testing of the quality of water and bottom sediments.

2. Study area

Lake Słosineckie Wielkie (53°56.3' altitude and 17°00.0' longitude) is situated in the north of Poland in the Bytowskie Lake District. It is adjacent to the Polanowska Upland to the north, the alluvial Charzykowska Plain in the Brda basin to the south and the Kaszubskie Lake District to the east. The upper Wieprza Valley runs through the middle of it. In the Bytowskie Lake District a significant number of lakes is present, although they are mostly small ones. The biggest ones include Bobięcino Wielkie (ca 5 km^2 in area, 48 m deep), Mausz (ca 4 km^2 in area, 45 m deep) and Kłączno (ca 2 km^2 in area, 17 m deep) (Kondracki 1994).

Lake Słosineckie Wielkie has a surface of 41.5 ha and is situated at the altitude of 157.8 meters above sea level. The lake dimensions are as follows: maximum depth – 8.1 m, average depth – 3 m, the length of the

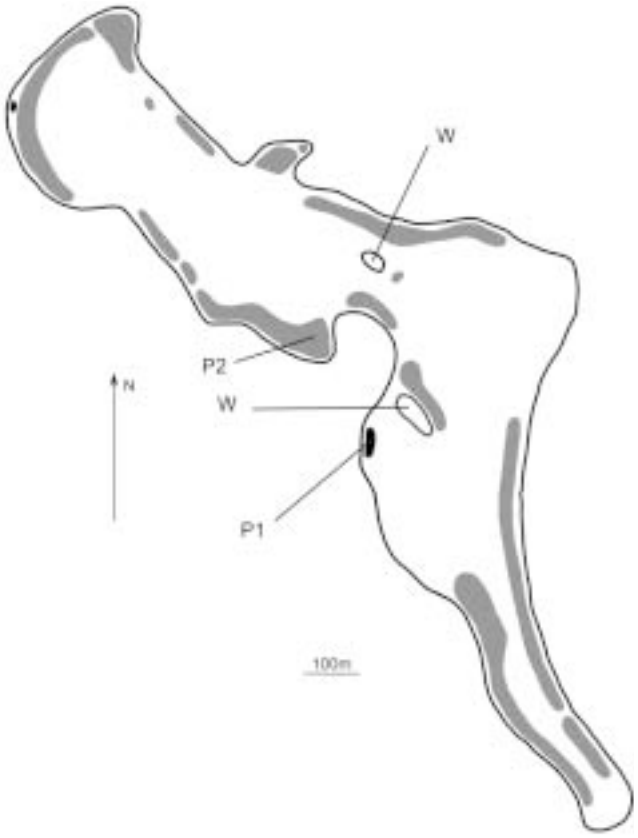

Fig. 1. Distribution of the populations of *Stratiotes aloides* in Lake Słosineckie Wielkie
Explanations: W – island; P1 – emergent form; P2 – submerged form

shore line 4725 m, the maximum length 1800 m and the maximum width 370 m. Water has a pH value of 8 with calcium content of 35 mg/l (Jańczak 1997).

In comparison to the Baltic littoral plains and alluvial plains to the south, the climate is on average 1°C colder (yearly average 6-7°C), while precipitation is heavier by more or less 100 mm (yearly average 600-700 mm) (Kondracki 2000).

3. Material and methods

The material comprising of 40 specimens and 909 leaves of *Stratiotes aloides* was collected in July and August 2004 from Lake Słosineckie Wielkie. The first group (20 specimens) grew above the water surface and reproduced both generatively and vegetatively. The second group (20 specimens) grew exclusively below the water surface and reproduced vegetatively. The emergent and submerged forms of *S. aloides*, as well as water and sediment samples, have been collected from two research areas accordingly (Fig. 1).

In the collected fresh material, the following features have been determined: the diameter and length of the shoot, sex (for emergent specimens), number of generative and vegetative organs, the angle of the leaf top, leaf width measured 3 cm from the base, 10 cm from the base and 10 cm from the top, leaf area, cellular wall thickness, number of vascular bundles, and number of chloroplasts.

The plant material has been dried in an electrical dryer at a temperature of 80°C for the following 3 days, 8 hours a day. The dry mass of the plants and their organs has been determined. The samples of the fresh material has been fixed in FAA (70% ethanol – acetic acid – formalin) and then processed into paraffin. Cross

Fig. 2. The habit of the emergent (left) and submerged (right) forms of *Stratiotes aloides* occurring in Lake Słosineckie Wielkie

Table 1. Physical and chemical properties of the water and bottom sediment in the localities of *Stratiotes aloides* in Lake Słosineckie Wielkie (July, 2004)

	Water						Sediment	
	Turbidity mg SiO2 l^{-1}	pH	Total N mg l^{-1}	Total P mg l^{-1}	Ca mg l^{-1}	Mg mg l^{-1}	Total N % dry mass	Total P % dry mass
Emergent form locality	6	7.1	6.78	0.07	28.85	2.72	1.49	0.099
Submerged form locality	3	8.5	7.56	0.08	37.00	3.19	2.02	0.074

sections of leaves from FAA have been subjected to histochemical reactions to detect cell walls components: lignin (floroglucinol and hydrochloric acid), cellulose (Lugol's solution and sulfuric acid) and suberine (Sudan IV) (Gerlach 1972). Leaf surface (60 pieces) has been scanned with the help of image analyzer MET-ILO 8 and leaf cells were examined under a confocal and optical microscope. Chemical analysis of water and sediment has been conducted in the Laboratory of the Centre for Research and Environmental Control in Katowice.

4. Results

Two morphological forms of *S. aloides* have been identified in the lake – an emergent and submerged one. The emergent form occurs above or just underneath the water surface. It can flower and has rigid, brittle, short, dark green leaves (Fig. 2). The submerged form overgrows the bottom in the littoral belt that is 1.5-3.5 meters deep, and it has long, light green, brittle and spirally twisted mature leaves (Fig. 2). A map of the distribution of both *S. aloides* forms in Lake Słosineckie Wielkie has been made (Fig. 1). The submerged form, which overgrows the bottom of the littoral zone, is definitely dominant in the lake. The emergent form occurs in two patches situated in a different part of the lake. Water and bottom sediments have been sampled in the area of occurrence of emergent and submerged population. The environmental parameters of both localities differ only slightly (Table 1).

The analysis of variance ANOVA for 40 individuals of *Stratiotes aloides* has shown significant differences between emergent and submerged populations (Table 2). Those differences apply to the following characteristics: the number of offsets, sex, the plants diameter and length, the roots length, the number of generative shoots, buds, flowers, fruit and turions, the total mass of plants, the mass of the generative and vegetative parts, and turions mass. More offsets are present in the emergent form that has a specified sex. A comparison of mean variables in the both populations has been performed. The submerged forms don't have generative shoots and they don't bloom. Emergent forms produce more turions than submerged ones (Table 2a).

Analysis of variance for 909 leaves showed significant differences between leaves of the emergent and submerged populations (Table 2b). Those differences concern the following characteristics: leaf apex angle, leaf length, leaf width measured 3 cm and 10 cm from

Table 2. The comparison of the variability of the emergent and submerged form of *Stratiotes aloides* in Lake Słosineckie Wielkie

Features	Emergent form	Submerged form	F	p
a. The variance ANOVA for 40 specimens				
Plants diameter [in cm]	72.35	111.12	211.644	0.000
Plants length [in cm]	102.00	133.65	55.403	0.000
Roots length [in cm]	66.70	80,50	15.399	0.000
Number of generative shoots	4.35	0.00	154.469	0.000
Number of buds	1.65	0.00	9.191	0.004
Number of flowers	1.00	0.00	15.833	0.000
Number of fruits	1.65	0.00	16.027	0.000
Number of turions	3.10	1.20	5.837	0.020
Number of offsets	1.35	0.15	16.531	0.000
Total dry mass of plant [in gm]	20.774	14.333	9.910	0.003
Generative dry mass of plant [in gm]	0.154	0.000	32.028	0.000
Vegetative dry mass of plant [in gm]	20.619	14.333	9.469	0.003
Turion dry mass [in gm]	0.114	0.003	15.180	0.000
b. The variance ANOVA for 909 leaves				
Leaf apex angle [in degrees]	18.07	10.95	2990.814	0.000
Leaf length [in cm]	29.15	47.72	2336.661	0.000
Leaf width 3 cm from the base [in mm]	13.37	9.70	1392.346	0.000
Leaf width 10 cm from the base [in mm]	14.51	11.97	424.257	0.000
Leaf width 10 cm from the top [in mm]	14.09	9.01	2509.087	0.000

Table 3. The comparison of prickles characteristics on the mature leaves of the emergent and submerged form of *Stratiotes aloides* (N=20)

	Leaves of the emergent form (length 26-29 cm)	Leaves of the submerged form (lenght 37-51 cm)
Number of prickles per leaf	121.2	152.8
Number of prickles per 1 cm^2 of leaf blade	4.46	3.40
Angle of prickles divergence from the longitudinal axis of the leaf	38°	17°

the base, and also 10 cm from the top. The emergent form leaves are straight with the tips of blades slightly tilted backward. The submerged form leaves are spirally twisted. The angle of the twist is on average 238.7°. The leaves of the emergent form have entire margins, while the margins of the submerged form are undulate.

Both forms have prickle-hairs on the edges of their leaf blades. The differences between the number, shape and angle of prickles divergence from the longitudinal leaf axis were shown (Table 3).

Twenty mature leaves of the emergent and submerged form have been scanned. The obtained images were subjected to a decimal-to-binary conversion and measurements of their surface area were taken. The coefficients of the leaves shape were determined with the use of Met-Ilo 8 image analyzer. The differences in the leaf blade area, perimeter and shape coefficient were revealed (Table 4).

gent form. The coefficient of the emerged form leaf shape is 35% higher, confirming the fact that the emerged leaves have wider angle of leaf apex. The differences between leaves of the emergent and submerged forms of *S. aloides* were shown in the Table 5.

The number of chloroplasts in leaf epidermis in the emergent and submerged form of *S. aloides* is similar. The average amount of chloroplasts in one cell is 18. Observations with the use of a confocal microscope has shown that chloroplasts in the emergent form leaf epidermis are distributed in a different manner than in the submerged form leaf cells. In the leaves of the emergent form chloroplasts are localized in the vicinity of anticlinal walls (Fig. 3), and in the submerged form they adjoin periclinal walls (Fig. 4).

The adaxial epidermis of the basal part of the emergent form leaf is characterized by both longitudinal and lateral arrangement of the cells in relation to the longi-

Table 4. The comparison of the area, perimeter and shape coefficient of the emergent and submerged form leaves of *Stratiotes aloides* L. (N=20)

	Mature leaf of the emergent form (length 21 cm)	Mature leaf of the submerged form (length 41 cm)
Blade area in mm^2	4381	5370
Perimeter in mm	693	902
Shape coefficient	0.20	0.13

The surface area of the mature leaves of the submerged form is 18.4% larger and the leaf perimeter is 23.1% longer than for the mature leaves of the emer-

tudinal leaf axis as well as the occurrence of paracytic stomata. Cell walls of the emergent form leaf epidermis (1.7 µm) are on average twice as thick as in the

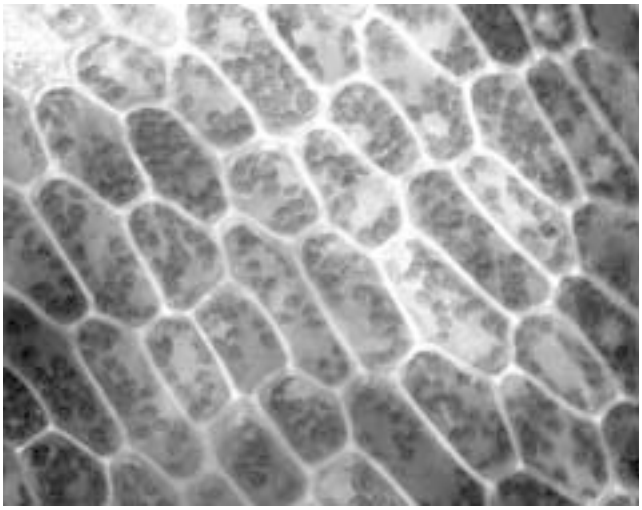

Fig. 3. Epidermal cells of the emergent form of *Stratiotes aloides* leaves (600x)

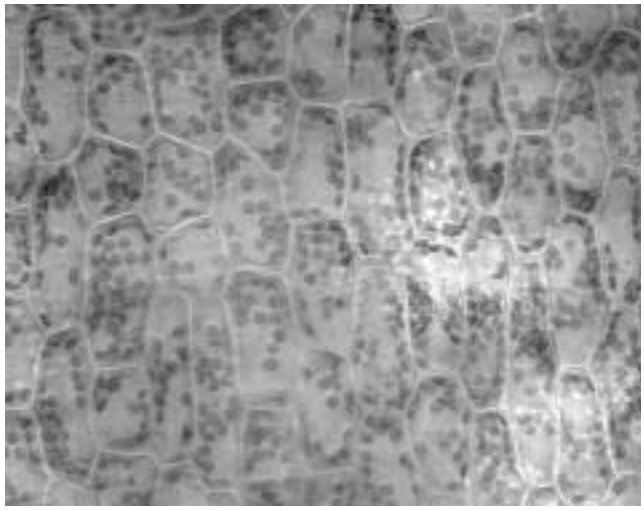

Fig. 4. Epidermal cells of the submerged form of *Stratiotes aloides* leaves (600x)

submerged form (0.7 µm) (Figs. 3 and 4). The adaxial epidermis of the basal part of the submerged form leaf shows longitudinal cell arrangement, concordant with longitudinal leaf axis, as well as the lack of stomata.

Cross-sections of the emergent and submerged form leaves differ depending on the distance from the leaf base. The number of big and medium vascular bundles in the emergent and submerged leaf is the same. That is, one big bundle in the main leaf vein and 6 bundles in the flattened lateral parts of the leaf. In the emergent form leaf there are much more fine bundles in comparison with the submerged form. The outside ring of reversed bundles occurs in leaves of both the emergent and submerged forms. Vascular bundles are collateral. Small bundle in the adaxial part of the emergent form leaf contains a single xylem element (25 x 37 µm in dimension) and many fine phloem elements. The small bundle in the upper part of the submerged form leaf consists of 3 xylem elements (18 x 20 µm in dimension) and many fine phloem elements.

The results of histochemical reactions in *S. aloides* leaves pointed that both *S. aloides* forms lack suberine and cellulose. When staining for lignin, the red coloration of vessels in the leaves of emergent form and lack of such coloration in leaves of submerged form has been found.

There were also observed significant differences in the aerenchyma structure of both forms. Examination of sections taken 10 cm away from the leaf bases showed that in the emergent plants the cells of spongy mesophyll surrounding the intercellular spaces have oval or round shape. There are 8-9 cells encircling a single intercellular space. Spaces dimensions amount to 90 x 120 µm. In contrary, the submergent form has tetragonal or hexagonal cells and there are 14-18 of cells per single intercellular space. Spaces dimensions are 200 x 300 µm.

5. Discussion

In Poland four ecotypes of *S. aloides* have been distinguished in the Masurian Lake District: the weak form of water soldier and the ecophenes of the early emerging, the late emerging and the submerged water soldier which are included in the shapely form (Kornatowski 1985). The maximum leaves length in the emergent and submerged form of *S. aloides* was 45 cm (Kornatowski 1983/84). While in Lake Słosineckie Wielkie the maximum leaves length of emergent *S. aloides* form was 38 cm and the submerged form 63.6 cm. The maximum leaves length of Swedish *S. aloides* was accordingly: 35 cm for the emergent form, and 36.9 cm for the submerged one (Renman 1989). The comparison of maximum length of leaves shows that in Lake Słosineckie Wielkie it is the submerged form that has the largest leaves.

The roots length of the early emerging form is 1 m, for the late emerging form 1.5 m, and in the case of weak form 1 m (Kornatowski 1983/84). In Lake Słosineckie Wielkie the roots length of the emergent form is on average 66.7 cm and for the submerged form 80.5 cm. The roots length of Swedish *S. aloides* is accordingly: 99.7 cm for the emergent form and 44.4 cm in the case of the submerged one (Renman 1989). Roots of the submerged form in Lake Słosineckie Wielkie are longer than in the emerged form, contrary to *S. aloides* in Sweden and in the Masurian Lake District. The emergent form of Swedish *S. aloides* has twice as many roots as the submerged form and they are twice as long (Renman 1989). In Lake Słosineckie Wielkie the emergent and submerged forms have 8-10 roots. The number of roots of the early emerging form is 5-14 and for the late emerging and weak forms 4-10 (Kornatowski 1983/84).

The number of turions and offsets in the case of Swedish *S. aloides* is higher in the emergent form (Renman 1989). It has been confirmed by the research

Table 5. Descriptive morphological and anatomical differences between the leaves of the emergent and submerged form of *Stratiotes aloides* in Lake Słosineckie Wielkie

Leaf features	Emergent form	Submerged form
Length	Shorter	Longer
Width	Larger	Smaller
Blade area	Smaller	Larger
Perimeter	Smaller	Larger
Shape coefficient	Higher	Lower
Apex angle	Wider	Narrower
Axis	Straight	Twisted
Margin	Entire	Undulate
Elasticity	Rigid	Flexible
Color	Dark green	Light green
Cross section	V-shape	I-shape
Number of small vascular bundles	Many	Few
Cell walls of leaf epidermis	Thick	Thin
Chloroplasts	Near antyclinal walls of leaf epidermis	Near peryclinal walls of leaf epidermis
Stomata	Present	Absent
Aerenchyma	Small air spaces	Big air spaces

on Lake Słosineckie Wielkie. Habitats of the emergent and submerged form in this lake have a similar content of nutritious substances, therefore they are not the reason for the differences in plant size. The submerged form in Lake Słosineckie Wielkie attains larger size than the emergent form, thus it is not the weak form of *S. aloides* described by Kornatowski (1983/84).

De Geus-Kruyt's and Segal's (1973) observations concerning dimorphism of *S. aloides* has been confirmed. Significant predominance of the submerged form over the emergent one in regards to its size has been found in Lake Słosineckie Wielkie. Erixon (1979) and Ozimek (1997) have made similar observations. A lack of florescence in the submerged form of *S. aloides* has been confirmed (Erixon 1979; Cook & Urmi-König 1983).

Similar mechanism of distribution of chloroplasts in epidermis cells that has been observed in the *Stratiotes* leaves has been also observed in *Lemna trisulca*. In the *Lemna* case the arrangement of chloroplasts changed depending on whether the plant was in full sun or in a shady place (Jacob *et al.* 1983). Light reaching the submerged forms of *S. aloides* growing at a depth of 1.5-3 m has smaller intensity. So chloroplasts in leaves of submerged form of *S. aloides* behave similarly to chloroplasts in *Lemna trisulca* in a shady place.

S. aloides leaves are spirotristichous, in a rosette (Tomlinson 1982), rigid, sessile, linear or narrowly triangular, with spinous – serrate margins (Cook 1998). Emergent leaves are thick, rigid, brittle, dark green, with well-developed spines; submerged leaves are thin, flaccid but brittle, light green, with weak spines (Cook & Urmi-König 1983). It's been established that the underwater leaves are longer than the floating ones (Cook & Urmi-König 1983), but the researches do not describe the underwater forms of *S. aloides*. Among others, Arber (1921), Ancibor (1979) and Tomlinson (1982) have dealt with the structure of *Stratiotes* leaves. Contradictive facts on the stomata distribution in *Stratiotes* leaves have been published (Cook & Urmi-König 1983). Our research has confirmed the occurrence of stomata on the adaxial surface of the emergent form leaves and their lack on the submerged form leaves. The presence of prickle-hairs on the edges of *Stratiotes* leaves observed by Cook and Urmi-König (1983) has been confirmed as well. Tomlinson (1982) specified the number of bundles in *Stratiotes* leaves. Types and structure of bundles occurring in *Stratiotes* leaves have been confirmed as well as the characteristic outside ring of reversed bundles described by Solereder (1913) and Arber (1921).

Summarizing, *S. aloides* is a perennial producing emergent and submerged forms, which differ in many morphological and anatomical characteristics. Our research has shown some differences as yet not described. Distinguishing two forms of *S. aloides* is not contradictory to distinguishing four morphological ecotypes because in the former case a morphological and anatomical criteria have been used while in the latter the phenological ones. Some of the studied characteristics might be determined only on fresh plant material, such as e.g.: leaf twisting or colour because they don't preserve well in herbarium material. The distinguished morphological and anatomical features may find application in *Stratiotes* genus taxonomy.

References

Ancibor E. 1979. Systematic anatomy of vegetative organs of the Hydrocharitaceae. Bot. J. Linn. Soc. 78: 237-266.

Arber A. 1921. Leaves of *Helobiae*. Bot. Gaz. 72: 31-32.

Cook C. D. K. 1998. Hydrocharitaceae. In: K. Kubitzki (ed.). The familie and genera of vascular plants. IV. Flowering plants. Monocotyledons. Alismatanae and Commelinanae (except Graminae), pp. 235-260. Springer Verlag, Berlin-Heidelberg-New York.

Cook C. D. K. & Urmi-König K. 1983. A revision of the genus *Stratiotes* (Hydrocharitaceae). Aquatic Bot. 16: 213-249.

De Geus-Kruyt M. & Segal S. 1973. Notes on productivity of *Stratiotes aloides* in two lakes in the Nederlands. Pol. Arch. Hydrobiol. 20: 195-205.

Erixon G. 1979. Population ecology of a *Stratiotes aloides* L. stand in a riverside lagoon in N. Sweden. Hydrobiologia 67: 215-221.

Gerlach D. 1972. Zarys mikrotechniki botanicznej. 296 pp. PWRiL, Warszawa.

Glück H. 1936. Pteridophyten und Phanerogamen unter gleichzeitiger Berücksichtigung der wichtigsten Wasser- und sumpfgewächse des ganzen Kontinents von Europa. In: A. Pascher (Hrsg.). Die Süßwasser-Flora Mitteleuropas, 15, pp. xx+486. G. Fischer Verlag, Jena.

Jacob F., Jäger E. J. & Ohman E. 1983. Kompendium der Botanik. 494 pp. VEB Gustav Fisher Verlag, Jena.

Jańczak J. 1997. Atlas jezior Polski. T. II. Jeziora zlewni rzek Przymorza i dorzecza dolnej Wisły. 256 pp. Instytut Meteorologii i Gospodarki Wodnej. Bogucki Wyd. Nauk., Poznań.

Kondracki J. 1994. Geografia Polski. Mezoregiony fizyczno-geograficzne. 339 pp. PWN, Warszawa.

Kondracki J. 2000. Geografia regionalna Polski. 440 pp. Wyd. Nauk. PWN, Warszawa.

KORNATOWSKI J. 1976. Dynamics of *Stratiotes aloides* L. development. Pol. Arch. Hydrobiol. 23(3): 365-376.

KORNATOWSKI J. 1979. Turions and offsets of *Stratiotes aloides* L. Acta hydrobiol. 21(2): 185-204.

KORNATOWSKI J. 1983/84. Morphological forms of the water soldier (*Stratiotes aloides* L.). Acta hydrobiol. 25/26: 145-156.

KORNATOWSKI J. 1985. Phenological and morphometrical differentiation of the water soldier (*Stratiotes aloides* L.). Acta hydrobiol. 27: 33-47.

MĄDALSKI J. 1938. O owocujących okazach *Stratiotes aloides* L. w Polsce. Acta Soc. Bot. Pol. 15(3): 245-249.

OZIMEK T. 1997. Submerged macrophytes as a substrate for *Dreissena polymorpha* (Pall.) in five lakes of the Jorka river watershed. Pol. Arch. Hydrobiol. 44: 445-455.

PODBIELKOWSKI Z. & TOMASZEWICZ H. 1982. Zarys hydrobotaniki. 530 pp. PWN, Warszawa.

RENMAN G. 1989. Life history of two clonal populations of *Stratiotes aloides* L. Hydrobiologia 185: 211-222.

SMOLDERS A. J. P., DEN HARTOM C. & ROELOFS J. G. M. 1995a. Observations on fruiting and seed-set of *Stratiotes aloides* L. in the Nederlands. Aquatic Bot. 51: 259-268.

SMOLDERS A. J. P., DEN HARTOM C. & ROELOFS J. G. M. 1995b. Germination and seedling development in *Stratiotes aloides* L. Aquatic Bot. 51: 269-279.

SOLEREDER H. 1913. Systematisch-anatomische Untersuchung des Blattes der Hydrocharitaceen. Beihefte zum Botanischen Centralblatt 30(1): 24-104.

STRZAŁEK M. 2004. Zielony wojownik w natarciu, czyli osoka w ekosystemach wodnych. Wiad. Ekol. 1(2): 83-105.

SZAFER W., KULCZYŃSKI S. & PAWŁOWSKI B. 1986. Rośliny polskie. xxxi+1020 pp. PWN Warszawa.

TOMA C. 2001. The development of *Stratiotes aloides* fruit. In: J. SALAJ, B. OBERT & T. SALAJ (eds.). From Gametes to Embryos. 10th International Conference of Plant Embryology, p. 57. Institute of Scientific and Technical Information for Agriculture Nitra, NOI Publishing, Slovak Republic.

TOMLINSON P. B. 1982. *Helobiae* (*Alismatidae*). In: C. R. METCALFE (ed.). Anatomy of Monocotyledons, Vol. 7, pp. 126-197. Clarendon Press, Oxford.