

## On systematics and history of the Bulgarian representative of genus *Isoetes* L.

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**Abstract.** For the first time the morphology of fossil and recent macro- and microspores of *Isoetes* L. was subjected to SEM studies and it was established that the spores belong to one and the same species, *Isoetes lacustris* L. Data of spore-pollen and macrofossil analyses have shown that in Late Quaternary and Early Holocene (11800-7000 BP) *Isoetes lacustris* was widely spread in Bulgaria. The shrinking of its area during Holocene was related to gradual overgrowth and drying up of the water basins inhabited by it.

**Key words:** *Isoetes*, spore morphology, Late-Quaternary history, Bulgaria.

### Introduction

During Late Quaternary and Holocene a number of aquatic plants in Europe manifested radical changes in their area. Contrary to the majority of tree taxa, whose rapid distribution and occupation of new terrains during Holocene had expanded their areas, changes in aquatic plants related to loss of territories and shrinking of areas (L a n g 1994).

In Bulgaria genus *Isoetes* L. has aroused not only the interest of paleobotanists, but also of florists and taxonomists. It is noted in Bulgarian and foreign botanical literature that this genus is represented now in Bulgaria by a single species reported as *Isoetes lacustris* L. (H a y e k 1927; S t o j a n o f f & S t e f a n o f f 1933, 1948; S t e f a n o f f 1943; A c h t a r o v 1963; J e r m y 1964; A n d r e e v 1992), *Isoetes setacea* L a m . (V e l c h e v & B o n d e v 1984; K o c h e v & J o r d a n o v 1981; K i t a n o v & K i t a n o v 1990), or *Isoetes echinospora* D u r i e u (J a l a s & S u o m i n e n 1972; G r e u t e r & al. 1984; F e r r a r i n i & al. 1986). Identification of the species of genus *Isoetes* has always been extremely difficult, mainly owing to their very similar external morphology (H i c k e y & al. 1989). Most taxonomic classifications in this genus have been based mainly on the macrospore sculpture (P f e i f f e r 1922; K o t t & B r i t t o n 1983). So far, such studies have not been done in Bulgaria.

The absence of an uniform taxonomic standpoint on the now existing representative of the genus in Bulgaria and the fossil macro- and microspores of *Isoetes* discovered in the Late Quaternary sediments of some lakes in the Pirin Mts, have determined the purpose of this investigation: 1. To study the sculpture specificities of recent and fossil macrospores with a Scanning Electron Microscope (SEM). To establish on the basis of this analysis whether it was one and the same species and which species occurred in the area in the past and now; 2. To trace out the Late

Quaternary distribution of the species in Bulgaria on the basis of data obtained from spore-pollen and macrofossil analyses.

### **Taxonomic treatment and contemporary distribution of genus *Isoetes* L. in Bulgaria**

*Isoetes* L. is a cosmopolitan genus of heterosporous lycopsids comprising approximately 150 species, including aquatic and terrestrial forms (T a y l o r & H i c k e y 1992). Spore size and ornamentation have been often the major characters used in distinguishing or determining *Isoetes* species (P f e i f f e r 1922; K o t t & B r i t t o n 1983). In spite of considerable variation of the sporoderm morphology (H i c k e y 1986), the characteristics of macrospores and more seldom of microspores have been used in the identification of species in many floras and regional studies (for instance, B e r t h e t & L e c o c q 1977).

Genus *Isoetes* was mentioned for the first time for Bulgaria with its species *Isoetes lacustris* L. by H a y e k (1927). It was featured under the same name in the floras of S t o j a n o f f & S t e f a n o f f (1933, 1948), of A c h t a r o v (1963), as well as in the studies of J e r m y (1964) and A n d r e e v (1992). Under the name *Isoetes setacea* L a m. the species was given in the flora by S t o j a n o f f & al. (1966), where *I. lacustris* auct. Fl. Bulg., non L. was given as its synonym. J a l a s & S u o m i n e n (1972) and J e r m y & A k e r o y d (1993) maintained a different opinion, excluding *I. lacustris* from the Bulgarian flora and including instead *I. echinospora* D u r i e u. In the works of G r e u t e r & al. (1984) and F e r r a r i n i & al. (1986) Bulgaria was also added to the area of *I. echinospora*.

Contemporary distribution of *I. echinospora* and *I. lacustris* in Europe takes place mainly in the northern part of the continent, with local occurrences of both species in the south. The area of *I. setacea* includes Southwest Europe (J a l a s & S u o m i n e n 1972; J e r m y & A k e r o y d 1993).

In Bulgaria *Isoetes* is distributed only in the Pirin Mts, Southwest Bulgaria (Fig. 1). It was reported in a number of glacial lakes in those mountains: Banderitsa, Popovo, Todorino, etc. lake groups (K o c h e v & J o r d a n o v 1981). The lakes are chiefly oligotrophic, formed mainly on Precarbon granites (B o y a d j i e v 1959; I v a n o v & S o t i r o v 1964).

### **Material and methods**

Fossil and recent macrospores and recent microspores of *Isoetes* were subjected to SEM analysis. The spores were collected in two lakes of the Popovi Ezero lake group: lake Popovo Ezero-1 (Papazgyol, 2263 m a.s.l.) and lake Popovo Ezero-6 (2185 m a.s.l.).

The fossil macrospores were separated in the analysis of plant macrofossil remains obtained from a 250 cm sediment core taken with a Dachnowski corer from the peripheral part of lake Popovo Ezero-6. The laboratory treatment of lake sediment samples for a macrofossil analysis, the obtained results and the dating of sediments, which began to accumulate somewhere about 7000 BP, were described in detail in S t e f a n o v a & B o z i l o v a (1995). Macrospores from sedi-

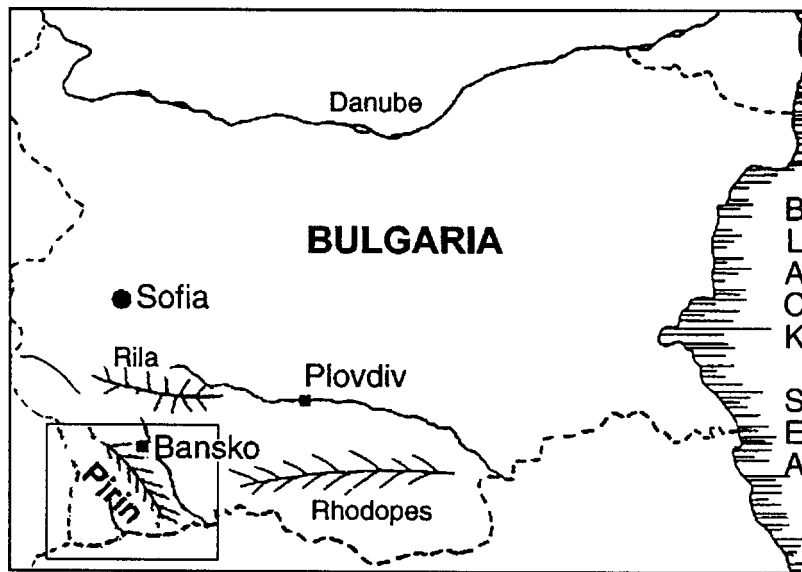


Fig. 1. Map of Bulgaria with contemporary distribution of *Isoetes* L.

ments dated to about 4000 BP, 3500 BP and 3000 BP were subjected to a SEM analysis.

The recent macro- and microspores were collected from lake Papazgyol in 1989 and 1998.

Spore sculpture was observed with JEOL SEM at 25 kV. The terminology used in the descriptions was after Hickey (1986), Tryon & Lugardon (1990), and Punt & al. (1994).

Twenty-five micro- and macrospores each were measured with light microscope, without the perisporium.

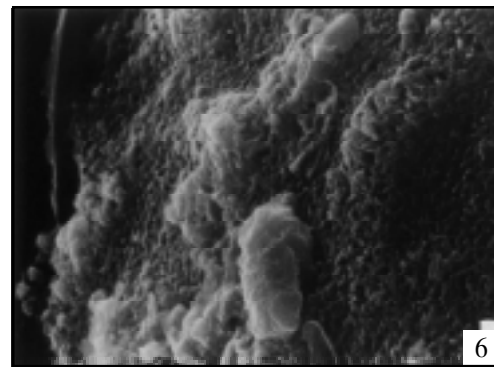
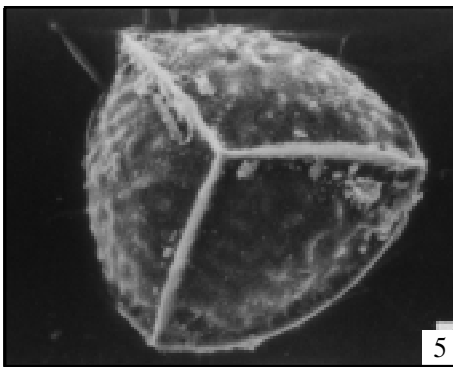
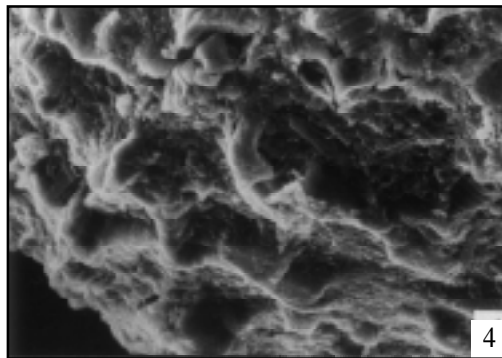
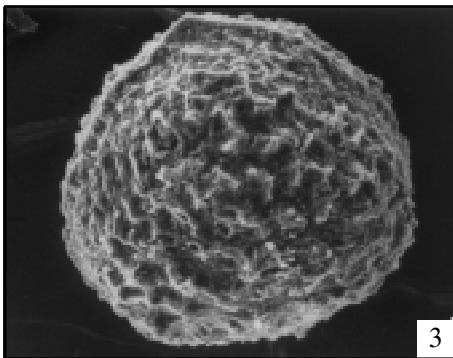
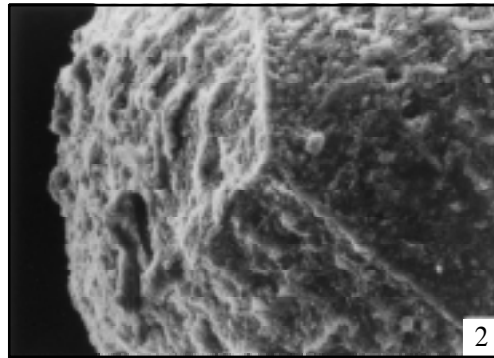
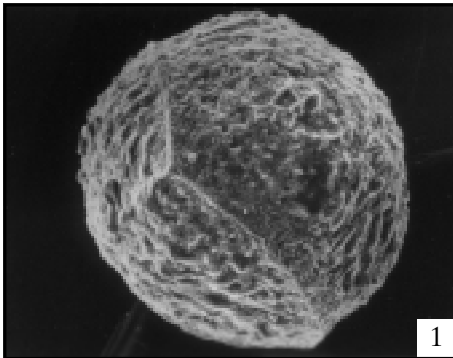
## Results and discussion

### Spore morphology

Fossil macrospores (Plate I): equatorial diameter 360-600  $\mu\text{m}$  (3000 BP), 470-530  $\mu\text{m}$  (3500 BP), 380-500  $\mu\text{m}$  (4000 BP), trilete, heteropolar, spheroidal or rounded triangular when viewed from the pole. Perisporium on the distal face with a dense system of short, irregular, not very distinct crests forming an incomplete cristate pattern (Plate I-3, 4). Proximal face with even less pronounced crests merging in spots into rugae (Plate I-1, 2, 5, 6). Laesura and equatorial ridge identical looking, narrow, straight, with or without a narrow strip of papillae in the adjacent zones (Plate I-1).

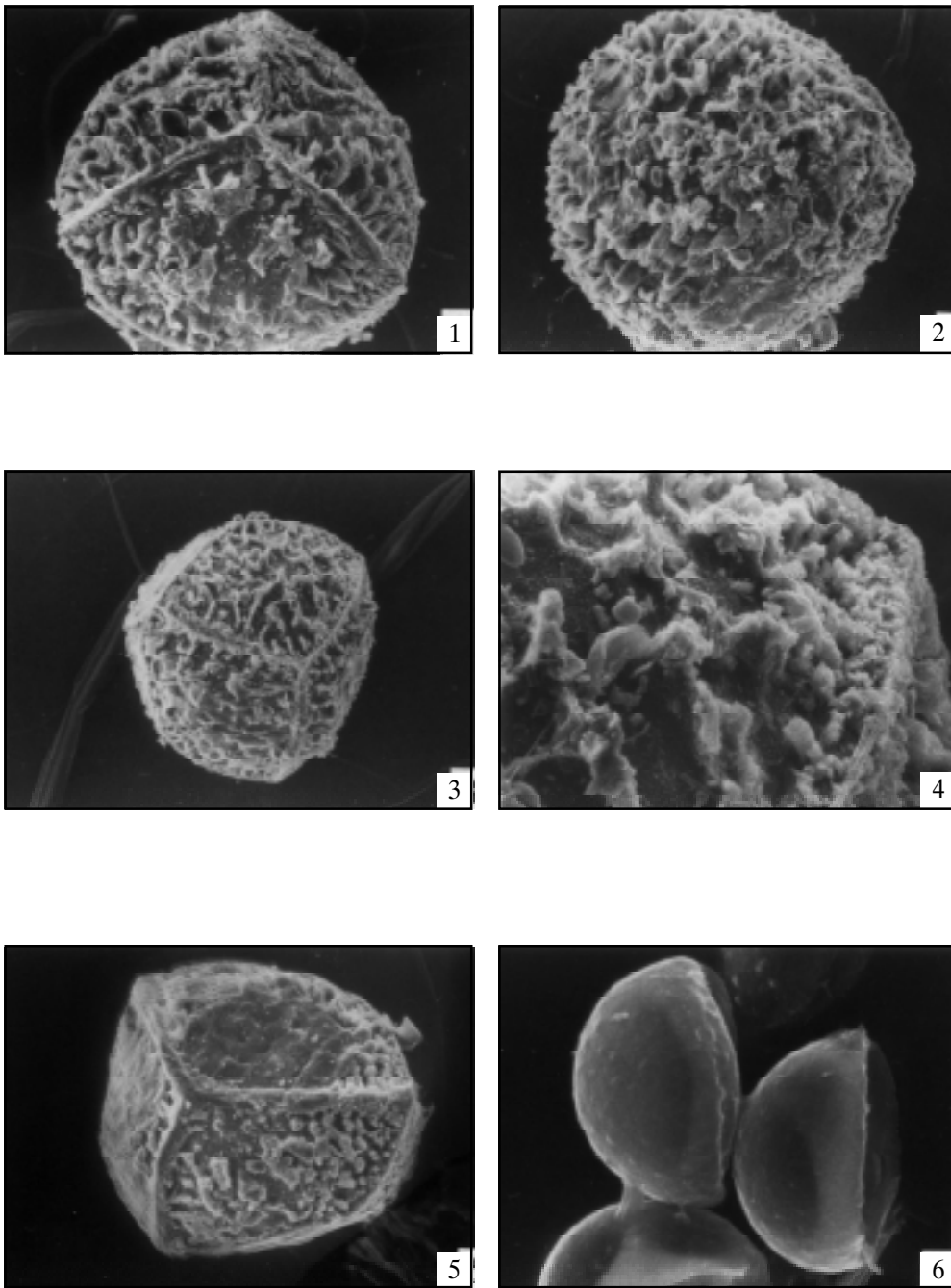
Recent macrospores (Plate II): equatorial diameter 448-572  $\mu\text{m}$ , trilete, heteropolar, radially symmetrical with a rounded triangular profile, when viewed from

**Plate I**



**Figures 1-6.** SEM photomicrographs of fossil *Isoetes* macrospores: 1. Macrospore (4000 BP), equatorial view, proximal face left,  $\times 150$ ; 2. Detail in equatorial region,  $\times 350$ ; 3. Macrospore (3500 BP), nearly distal view,  $\times 150$ ; 4. Surface detail,  $\times 500$ ; 5. Macrospore (3000 BP), proximal view,  $\times 200$ ; 6. Surface detail,  $\times 1500$ .

**Plate II**



**Figures 1-6.** SEM photomicrographs of recent *Isoetes* macrospores and microspores: 1. Macrospore, proximal view,  $\times 150$ ; 2. Macrospore, distal view,  $\times 150$ ; 3. Macrospore, near proximal view,  $\times 100$ ; 4. Macrospore, surface detail,  $\times 500$ ; 5. Macrospore, proximal view showing spore distortion,  $\times 150$ ; 6. Microspores nearly lateral view,  $\times 1500$ .

the pole. Perisporium on the distal face with relatively high crests, isolated or forming a network of irregular anastomoses, more or less flattened (Plate II-2). Proximal face with less dense and high ornamentation, occasionally distorted at the centre of proximal surfaces from the pressure into each other (Plate II-5), with a cristate or echinate perisporium with irregular, coarse, scarce spines (Plate II-1, 3, 5). Laesura and equatorial ridge identical looking, narrow and not very high, straight. There were much finer and denser spines or tubercles in the zones adjacent to the three ridges of the laesura and at the equatorial ridge (Plate II-1, 3, 4).

Recent microspores (Plate II): 35-42  $\mu\text{m}$  long, monolete, bilaterally symmetrical, with elliptical profile. Perisporium laevigate or with low, sparse, not very pronounced papillae or tubercles (Plate II-6). Laesura with not very prominent ridges (Plate II-6). Equatorial ridge missing.

Our SEM analysis of the sculpture specificities of fossil and recent *Isoetes* macrospores, as well as the observations with binocular and light microscope led us to the conclusion that the spores belonged to *Isoetes lacustris* L. (Table 1). Our data were similar to those published by Berthet & Lecocq (1977) and by Ferrarini & al. (1986). Missing was the echinate ornamentation characteristic of *I. echinospora* (Berthet & Lecocq 1977; Prada 1983; Kott & Britton 1983; Ferrarini & al. 1986; Britton & Brunton 1989, etc.), as well as the thick and distinct laesura and equatorial ridge, and tuberculate ornamentation of *I. setacea* (Berthet & Lecocq 1977; Prada 1983).

The sculpture of fossil and recent macrospores was similar, of cristate type. There were only slight differences in the perisporium sculpture of the material dated to 3000 BP. With fossil spores one should account for the conditions of their preservation in the sediments, which might be not equally favourable in time. A possibility of immature spores, which in the earlier stages of their development could show quite a different type of sculpture, should not be excluded too. The insignificant differences observed by us led us to the decision that it was the same taxon as with the more adult material.

The fact that the investigated material belonged to *I. lacustris* was also corroborated by the data about the size and character of the perisporium sculpture of recent microspores (Table 1). The microspores were larger than those of *I. echinospora* (24-30  $\mu\text{m}$ ) and *I. setacea* (28-35) reported by Berthet & Lecocq (1977), Prada (1983) and Ferrarini & al. (1986). The sculpture specificities of the perisporium of the investigated microspores - the absence of an equatorial ridge (as in *I. setacea*) and of spines (as in *I. setacea* and *I. echinospora*) - coincided with those reported by the above authors for *I. lacustris*.

### **Late Quaternary fossil finds of *Isoetes* in Bulgaria**

Unfortunately very few fossil finds of *Isoetes* have been evidenced in the country. Micro- and macrospores of *I. lacustris* were discovered in the Late Quaternary sediments in the Pirin Mts (Stefanova & Bozilova 1995; Stefanova in prep.) and in the Western Rhodopes (Huttunen & al. 1992).

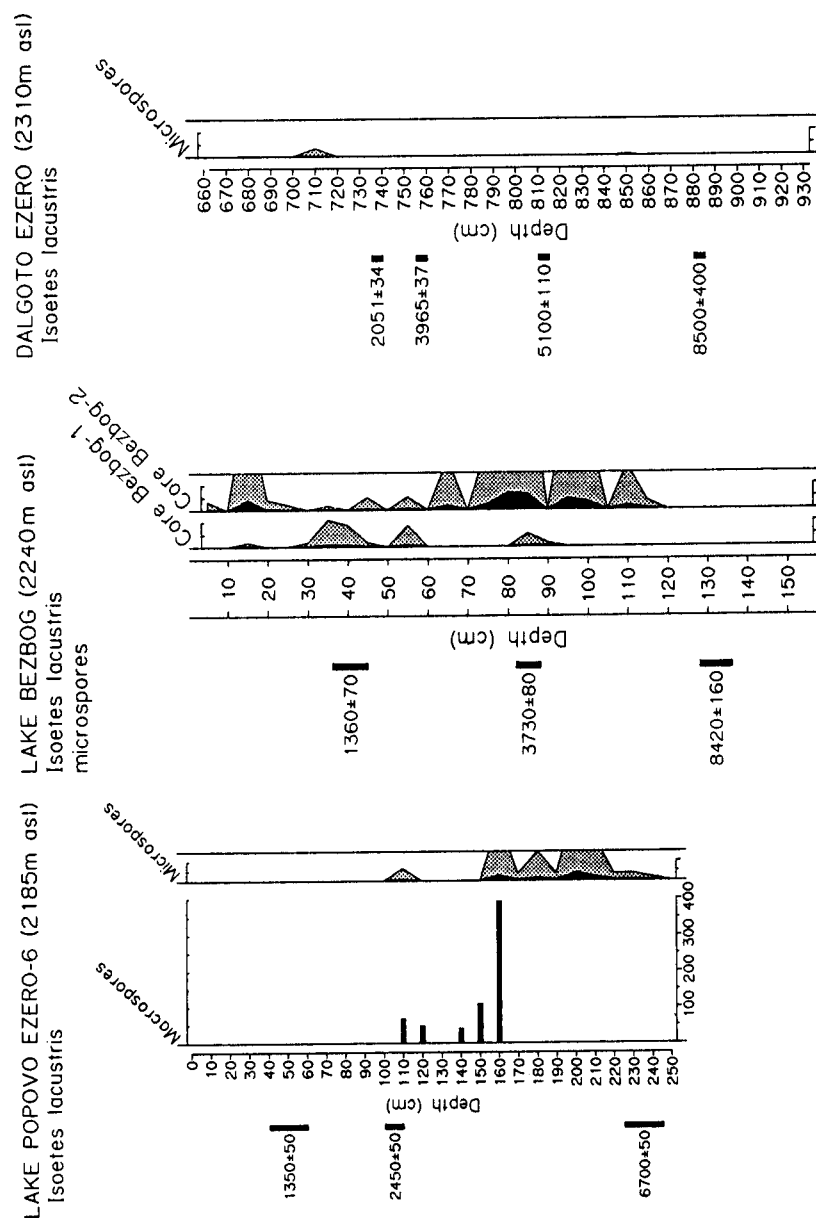
In the Pirin Mts the first microspores of *I. lacustris* (under 1% of the total sum AP+NAP) were established in sediments dated to about 7000 BP in three glacial

**Table 1.** Comparison of some characteristics and size parameters of spores from Bulgarian plants *Isoetes lacustris* L. with those found in literature for some other *Isoetes* species

	<i>I. lacustris</i>	<i>I. setacea</i>	<i>I. echinospora</i>	Material of Bulgarian origin
<b>MACROSPORES</b>				
Perisporium	Cristate, with crests forming a network of irregular anastomoses or with isolated short crests [A, B], furrowed by rugae on the distal face, echinate on the proximal face [C]	Tuberculate, with numerous tubercles [A, B]	Echinate, with numerous dense spines [A, B, C]	Cristate, with isolated, or forming a network of irregular anastomoses crests, in spots with coarse, scarce spines
Laesura	Narrow, low [A, B]	Broad, low [A, B]	Narrow, high [A, B]	Narrow, low
Equatorial ridge	Narrow, low [A]	Very prominent [A]	Slightly prominent [A]	Narrow, low
Equatorial diameter	450-630 $\mu\text{m}$ [A]	480-550 $\mu\text{m}$ [A]	380-550 $\mu\text{m}$ [A]	448-572 $\mu\text{m}$
	600-650 $\mu\text{m}$ [B]	560-580 $\mu\text{m}$ [B]	360-480 $\mu\text{m}$ [B]	
	509-577 $\mu\text{m}$ [C]		418-456 $\mu\text{m}$ [C]	
<b>MICROSPORES</b>				
Perisporium	Laevigate or finely grained, with low and sparse papillae [A, B, C]	Echinate, with short blunt spines [A, B]	Coarse, with dense spines [A, C]	Laevigate or with low, sparse papillae or tubercles
Laesura	With very prominent ridges [A]	Distinct, thick and prominent [A, B]	Strongly prominent [A]	With not very prominent ridges
Equatorial ridge	Missing [A]	Distinct and very prominent [A, B]	-	Missing
Length	40-50 $\mu\text{m}$ [A]	30-35 $\mu\text{m}$ [A]	25-30 $\mu\text{m}$ [A]	35-42 $\mu\text{m}$
	35-39 $\mu\text{m}$ [B]	28-33 $\mu\text{m}$ [B]	24-26 $\mu\text{m}$ [B]	
	38-44 $\mu\text{m}$ [C]		24-30 $\mu\text{m}$ [C]	

[A] - Berthet & Leccocq 1977 [B] - Prada 1983 [C] - Ferrarini et al. 1986

lakes: lake Popovo Ezero-6, lake Bezbog (Stefanova & Bozilova 1995), and lake Dalgoto Ezero (Stefanova in prep.). Paleoecological data showed that in the 6000-3000 BP period the species was best represented in the composition of the fossil aquatic vegetation (Fig. 2). In the spore-pollen spectra of



**Fig. 2.** Participation of *Isoetes lacustris* L. in fossil spore-pollen spectra of the investigated lakes in the Pirin Mts. The percentage content of microspores was calculated in relation to AP+NAP. Macrospores in the sediments of lake Popovo Ezero-6 are represented in absolute numbers for a volume of 50 cm<sup>3</sup>.



lake Popovo Ezero-6 the percentage content of microspores varied between 2-4%, and in those from lake Bezbog between 3-7%. The maximum development of the species was about 4000 BP. At that time the percentage content of microspores in lake Bezbog was 7% and in lake Popovo Ezero-6 4%, and the concentration of identified macrospores in the sediments of lake Popovo Ezero-6 reached its maximum. That was followed by a period of gradual restriction of *I. lacustris* in the composition of aquatic vegetation, and after 2400 BP the species disappeared from its composition in lake Popovo Ezero-6, while in lake Bezbog the participation of common quillwort was considerably reduced. In lake Dalgoto Ezero of the Banderishki Ezero lake group single microspores of *I. lacustris* were also discovered in the sediments dated approximately to 2000 BP. These were the last finds of this species, which is now missing from the lake aquatic flora (Stefanova in prep.).

In the Western Rhodopes single microspores of *I. lacustris* were identified in the Late Glacial sediments of the Kupena peat bog (1300 m a.s.l.) (Huttunen & al. 1992). These have been the earliest fossil finds reported so far. The species disappeared from the composition of fossil local flora after 7000 BP.

Paleorecords showed that in Late Quaternary and Early Holocene (11800-7000 BP) *I. lacustris* was wider spread in the country. Shrinking of its area, as was the case in the Western Rhodopes, was related to gradual overgrowth of the water basin inhabited by it and its transformation into a peat bog (Huttunen et al. 1992). In the Pirin Mts the disappearance of this species from the local flora of lake Popovo Ezero-6 about 2400 BP, as well as its reduced distribution in lake Bezbog also resulted from overgrowth of the lakes and shrinking of the open water surface. The transition of the type of sediments from fine into coarse detritus lake mud, and finally peat accumulation serves as an indicator for these changes (Stefanova & Bozilova 1995). It was much more difficult to explain the absence of common quillwort from the composition of contemporary local flora in lake Dalgoto Ezero. Contrary to the other two lakes, the peripheral peating processes in lake Dalgoto Ezero have been less prominent. That was evidenced also by the low development coefficient of its shoreline (Ivanov & Sotirov 1964). Along with that, in lake Zhabeshko Ezero (2322 m a.s.l.), situated several hundred meters away from lake Dalgoto Ezero and belonging to the same lake group, *I. lacustris* was represented on a mass scale. As single microspores were identified only in two samples of the investigated sediments from lake Dalgoto Ezero, it could be assumed that they were transferred there. There was evidence of an underground inflow of waters of the higher situated lake Zhabeshko Ezero into it (Ivanov & Sotirov 1964). Besides by water, distribution of spores of *I. lacustris* was also possible when herbivorous mammals used these plants for food (Camus & al. 1991).

The scanty data on fossil distribution of *I. lacustris* in Europe were summarized by Lang (1994) (Fig. 3). Judging by the Late Glacial finds of the species in the British Isles (Jessen & Farrington 1938; Vasari & Vasari 1968; Birks 1973) and in Southern Sweden (Berglund 1966; Digefeldt 1972), Lang (op. c.) assumed that the Late Glacial refugia of the common quillwort were chiefly in that part of the continent. Finds of *I. lacustris* in the Late Glacial sediments in the Western Rhodopes testified that this species had also refugia in Bulgaria. The data about the first emergence of *I.*

*lacustris* in the Pirin Mts and in the Massif Central Mts (France) were close in time, about 7500 BP (L a n g & T r a u t m a n n 1961). Similar abundance of the species in the Pirin Mts, somewhere between 6000-3000 BP, was also characteristic for other parts of Europe, with particularly close results from the investigations of Schwarzwald, Switzerland (L a n g 1955). Data from Bulgaria on the area shrink-

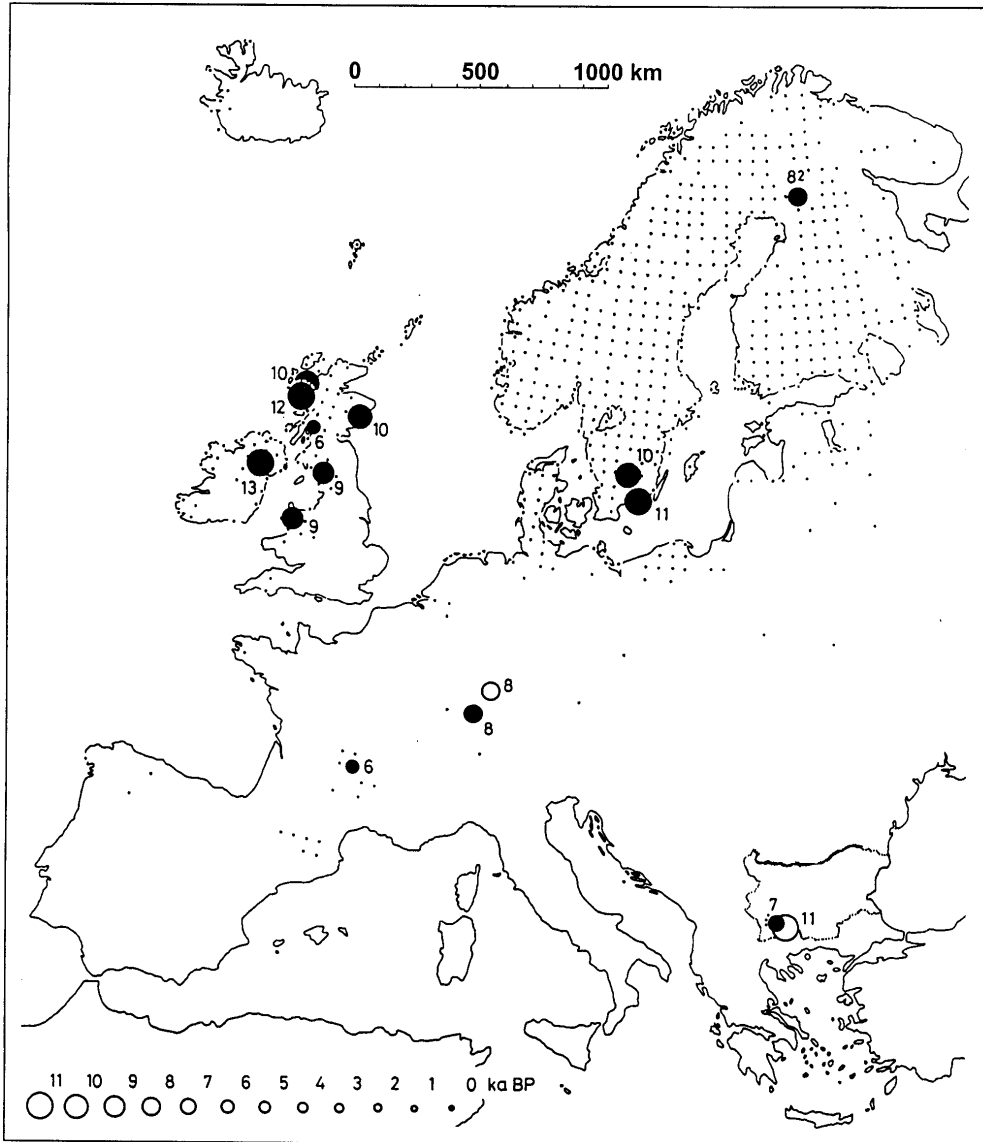


Fig. 3. Map of the contemporary area and fossil finds of *Isoetes lacustris* L. in Europe according to L a n g (1994), supplemented with data from Bulgaria. The circles and large black dots with numbers indicate the radiocarbon dating of finds; the large white circles indicate finds in localities without contemporary habitats; the large black dots indicate finds in localities with contemporary habitats; the small dots indicate contemporary area (after J a l a s & S u o m i n e n 1972).

ing of the common quillwort during Holocene coincide with those published for other parts of Europe (Lang 1994) and they support the explanation that this process was due to drying up of the water basins where the species used to live.

## Conclusions

The results from the SEM analysis of fossil and recent macrospores of genus *Isoetes* confirmed their belonging to one and the same species: *Isoetes lacustris* L.

In Late Quaternary and Early Holocene (11800-7000 BP) *I. lacustris* was wider distributed in the country: in the Pirin Mts and in the Western Rhodopes.

Finds of *I. lacustris* in the Late Glacial sediments in Bulgaria showed that it was distributed not only in the western parts of the Continent.

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