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Observations on the rare alga *Thorea hispida* (Thore) Desvaux (Rhodophyta) from Serbia

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Abstract – *Thorea hispida* (Thore) Desvaux (Rhodophyta) was first found in Serbia in the river Danube at the end of the 19th century (as *T. ramosissima* Bory de Saint-Vincent). It was not found again in Serbia until it was reported in 1996 in the river Beli Timok, Danube basin. *T. hispida* (as *T. ramosissima*) is included in the Red List of rare and threatened algae in some European countries, and its habitats in Serbia are critically endangered. This paper reports this species in the river Sava, a tributary of the river Danube. Both monosporangia and carposporangia were observed. Compared with other European populations, the Serbian population varied in morphology, particularly in the reproductive structures.

Distribution / Morphology / Reproduction / Serbia / *Thorea* / Threatened algae

Résumé – Observations sur *Thorea hispida* (Thore) Desvaux (Rhodophyta), algue rare trouvée en Serbie *Thorea hispida* (Thore) Desvaux (en tant que *T. ramosissima* Bory de Saint-Vincent) a été trouvée pour la première fois en Serbie, dans le Danube, à la fin du XIXe siècle. Cette algue n'a été retrouvée qu'en 1996 en Serbie dans la rivière Timok Blanche (bassin du Danube). *T. hispida* (syn. *T. ramosissima*) est inclus dans les Listes Rouges d'algues rares ou menacées de quelques pays d'Europe et son habitat en Serbie est sérieusement compromis. Cette espèce a été découverte, en 2008, dans la Save, affluent du Danube. Des monosporanges et des carposporanges ont été observés. Comparé à d'autres populations européennes, le matériel serbe montre une certaine variabilité des caractères morphologiques, particulièrement dans les structures reproductrices.

Algues menacées / Distribution / Morphologie / Reproduction / Serbie / *Thorea*

INTRODUCTION

The family Thoreaceae (Rhodophyta) is a family of freshwater red algae which is distinguished from the other members of the Batrachospermales most significantly in having multiaxial thalli (Starmach, 1977; Sheath *et al.*, 1993). Vis *et al*. (1998) suggested that Thoreaceae should be elevated to ordinal status but still considered as "incertae sedis" in the Florideophyceae. Müller *et al*. (2002)

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proposed the order Thoreales to accommodate the two recognized genera: *Thorea* and *Nemalionopsis*. Kumano (2002) noted that in *Thorea* the reproductive organs are formed on short branches (monosporangia, carposporangia and spermatangia), whereas in *Nemalionopsis* on long branches (just monosporangia). Additionally, the assimilatory filaments are sparsely branched and loose in *Thorea*, and densely branched and compact in *Nemalionopsis*.

Thorea has been reported from most continents (Europe, Africa, Asia, North America and South America, Australia) whereas *Nemalionopsis* is known only from a few localities in Asia and North America (Kumano, 2002).

Sheath *et al.* (1993) recognized only four species of *Thorea* worldwide: *T. hispida* (Thore) Desvaux, *T. violacea* Bory, *T. zollingeri* Shmitz and *T. clavata* Seto *et* Ratnasabapathy. Subsequently Entwisle & Foard (1999) described *T. conturba* from Australia, with a vegetative morphology somewhat intermediate between *Thorea* and *Nemalionopsis.* Carmona & Necchi (2001) regarded *T. violacea* as a synonym of *T. hispida*, but on the basis of molecular sequence data Müller *et al*. (2002) concluded that the two taxa should be retained as separate species although they are similar.

Populations of *Thorea* have been poorly documented in Serbia. Until recently *Thorea hispida* (as *T. ramosissima*) was known only from the river Danube, where it was collected in 1889 (Magnus, 1889) and 1919 (Vouk, 1953). More recent investigations carried out in those localities have not confirmed those findings (Simić *et al.,* 2007). This alga was rediscovered only 87 years later, in 1996, in the river Beli Timok, a tributary of the river Veliki Timok, which is part of the Danube basin (Simić & Ranković, 1998). This report was confirmed in this new locality in 2003 and 2005 (Simić *et al.,* 2007).

The aim of the present paper is to report new information on the occurrence, morphology and reproduction of this rare species in Serbia.

MATERIAL AND METHODS

Samples of *Thorea* were collected from two localities in Serbia (Fig. 1, Table 1). The first population was discovered in August 1996, at locality BT1 - the river Beli Timok, near Zaječar (N 43° 54′ 31″, E 22°18′ 26″). Samples of this alga from this locality were collected again in August 2005. A new location for *Thorea* was found in the river Sava, near Šabac (locality S1, N 44° 44′ 31″, E 19° 45′ 40″). Samples from this locality were collected in July 2008. Both localities are in the immediately adjactent to sites dedicated to sand and gravel exploitation for human purposes. The two earlier collections from the Danube (Magnus, 1989; Vouk, 1953), also shown in Fig. 1, are: D1 – the river Danube (N 44° 51′ 24″, E 20° 24′ 56″), and D2 – the river Danube (N 44° 49′ 93″, E 20° 30′ 53″) (Fig. 1, Tab. 1).

Samples were fixed immediately in 4% formaldehyde. The following environmental variables were measured for each sampling site: temperature (°C), current velocity ($cm{\cdot}s^{-1}$), pH, oxygen concentration ($mg{\cdot}l^{-1}$), saturation of oxygen (%), conductivity (measured with a digital conductometer of the HANA EP–3 type and expressed in $\mu S{\cdot}cm^{-1}$), depth (cm) and type of substratum. In addition, the percentage cover of the alga on the substratum was estimated for each sampling site (Table 1). Preserved material is stored in the collection of the Department of Biology and Ecology in Kragujevac, Serbia.

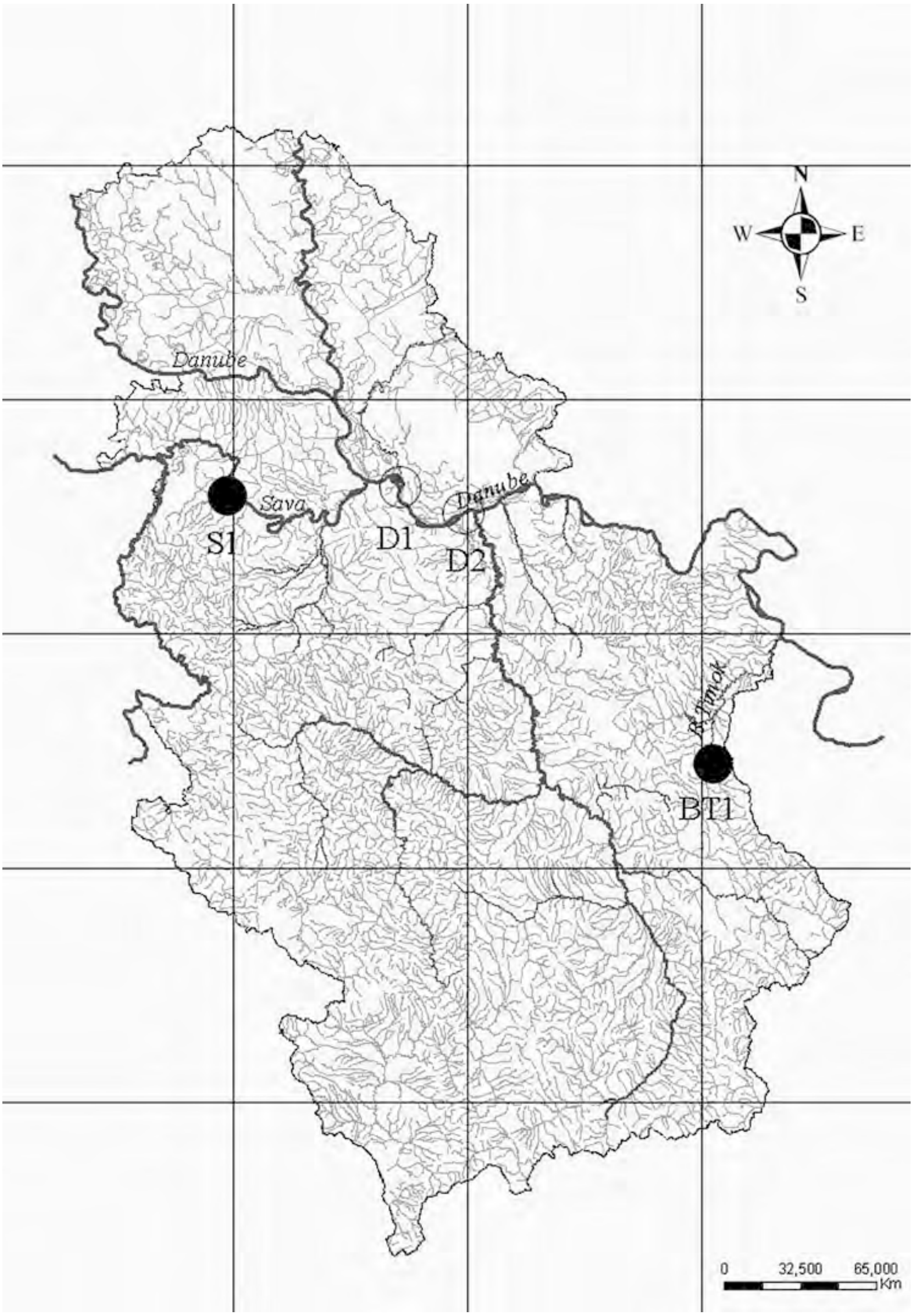


Fig. 1. Distribution of *Thorea hispida* in Serbia (locality D1 and D2 – the river Danube; BT1- the river Beli Timok and S1- the river Sava) (BT1 and S1: localities where populations were analyzed *T. hispida*).

Table 1. Characteristics of the river sections containing *Thorea hispida* populations in Serbia.

River	***River Danube*** (Magnus, 1989; Vouk, 1953)		***River Beli Timok*** (Simić & Ranković, 1998) (Simić et al., 2007)		***River Sava*** (new finding)
Locality*	D1 near Belgrade	D2 near Kovin	BT1 near Zaječar		S1 near Šabac
Years/month	1889	1919	1996/ August	2005/ August	2008/ July
Altitude (m)	117	67	145		65
Max. bed width (m)			10-20	10-20	650
Max. depth (m)			0.2-0.5	0.2-0.7	2
Substrate			rocks, gravel rotten trees	rocks, gravel	rocks, gravel
Percentage cover (%)			above 10%	20%	1%
Velocity ($cm \cdot s^{-1}$)			50.0.-70.0	50	70
Water temperature (°C)			24.0	23.0	22.5
Oxygen concentration ($mg \cdot l^{-1}$)			10.76	11	
Saturation of oxygen (%)			140	120	
pH			8.2	8.2	8.29
Conductivity ($\mu S \cdot cm^{-1}$)			480	450	429
Other macrophytes: macrolgae, Plants			*Cladophora* sp., *Potamogeton* sp.	*Cladophora* sp., *Potamogeton* sp.	*Cladophora* sp., *Potamogeton* sp.
Risk factors			exploitation of gravel	exploitation of gravel	exploitation of gravel

* According to Material and Methods.

Morphological characters considered to be of taxonomic importance were analysed. Microscopical analyses followed Sheath *et al.* (1993) and Carmona & Necchi (2001). In order to examine the frequency of secondary branches, the branch density for the whole plant (dense vs. sparse branching) was estimated (Carmona & Necchi, 2001), and the branches in a 30 mm length enumerated (Sheath *et al.*, 1993) to avoid the problems associated with irregularly branched thalli (Carmona & Necchi, 2001).

RESULTS AND DISCUSSION

Thorea hispida is a widespread species in different parts of the world (Europe, Africa, Asia, North America and South America) with different climatic characteristics, but the ecological conditions of habitats where the species is present are very similar (Sheath *et al.*, 1993; Wu, 1999; Carmona & Necchi 2001; Kumano, 2002). It was found in Serbia in July and August, in river sections where the riverbed is 10-20 m wide (the river Beli Timok, locality BT1), or 650 m (the river Sava, locality S1), at 0.2-2 m depth (Table 1). Thalli were found on stone and tree branches at non-shady sites, at 0.1-0.5m depth, water temperature 22.5-24°C, current velocity 50-70 cm·s^{-1}, pH 8.2, conductivity 429-480 µS.cm^{-1}, oxygen concentration 10.76 mg·l^{-1}, saturation of oxygen 140% (Table 1). Percentage coverage varied between 1% (river Sava, locality S1) and 20% (river Beli Timok, locality BT1, August 2005) (Table 1).

Characteristics of sexual and asexual structures in populations of *Thorea* in Serbia agreed with the general descriptions of spermatangia, carposporangia and monosporangia for *Thorea hispida* (Carmona & Necchi, 2001). The Serbian populations of *Thorea hispida* were characterized by a wide variation of morphological features (Tables 2, 3, Figs 2-18). Plants were multiaxial, moderately mucilaginous, plant length 15-50 cm, diameter 540-1800 µm, medula diameter 165-660 µm, assimilatory filament length 400-750 µm (Table 2, Figs 2, 3), with non-clavate assimilatory filaments (mean upper-to-lower cell diameter ratio 2.5-4.2), and sparsely branched (0-3, rarely 5 branchings) (Fig. 4) with secondary branches varying in frequence between 4 and 36 per 30 mm (Table 2, Figs 5, 6).

Both asexual (monosporangia) and sexual (only carposporangia) reproductive structures were present (Table 3, Figs 7-18).

Monosporangia were found on both localities in Serbia. They were usually borne singly, in pairs or in clusters of up to 3, with the branches of two cells arising from the basal cell of the assimilatory filament. Mature monosporangia were elliptical or ovoidal, 18 µm long and 9-14 µm in diameter (Table 3, Figs 7, 8). Monospore germination was also found (Figs 9, 10). The total length of monosporangial branches was about 30 µm (Fig. 8). The ratio between monosporangial branch length and assimilatory filament length was in this case always less then 0.1.

In the Serbian populations of *Thorea hispida* from the Beli Timok and Sava rivers an interesting and hitherto undescribed phenomenon was observed (albeit rarely), i.e. the creation of monosporangia from vegetative cells of long assimilators, both branched and unbranched (Figs 11-15). After their formation the newly formed monosporangia were detected terminally on long assimilators (length 400-600 µm) (Figs 11-12), or at short lateral branches (length of 30 to 100 µm), which originate from the top or middle of a long assimilatory filament (Figs 13-15). The ratio of monosporangial branches length to the sterile assimilatory filament length in this case was very variable (from 1 to less than 0.1). These monosporangia have similar shape and dimensions (length 18-22 µm, width 11-15 µm) to those formed at the base of assimilatory filaments at short branches.

Various authors (Starmach, 1977; John *et al.,* 1989; Sabater *et al.*, 1989; Dogadina *et al.,* 2009), reported *Thorea hispida* only with asexual reproductive structures and rare monosporangia, 8.6-30.0 µm in length, at the base of short assimilatory branches, single or in sori of 3(-8). Similar findings were made also by Kumano (2002). Female sexual reproduction organs were found in Europe for the first time in a population of *T. hispida* of Serbia (locality B1, year 1996) (Table 3, Figs 16-18). Carpogonia were formed relatively abundantly on the younger parts of primary and secondary branches. They were conical, 4.0-6.0 µm in diameter, 15 µm long, each having an elongate filiform and stright or slightly curved trichogyne, wich was up to 120 µm long, not extending above the assimilatory filaments (Table 3, Fig. 16). Carposporangia were borne in clusters of up to 8, terminally or subterminally on gonimoblast filaments (Figs 17, 18). They were club-shaped or obovoidal, with a granulated and dense content, 18.5-26 µm in length and 8-17 µm in diameter (Table 3). Gonimoblast filaments were composed of cylindrical cell of two kinds, *i.e.* long, prostrate and loosely arranged cells, with diffuse growth among and resembling medullary filaments; and short, erect, compactly arranged cells, with definite growth, forming fascicles and producing carposporangia.

Table 2. Vegetative characteristics of *Thorea hispida* populations from Serbia compared with known characteristics of some populations of this species from around the world.

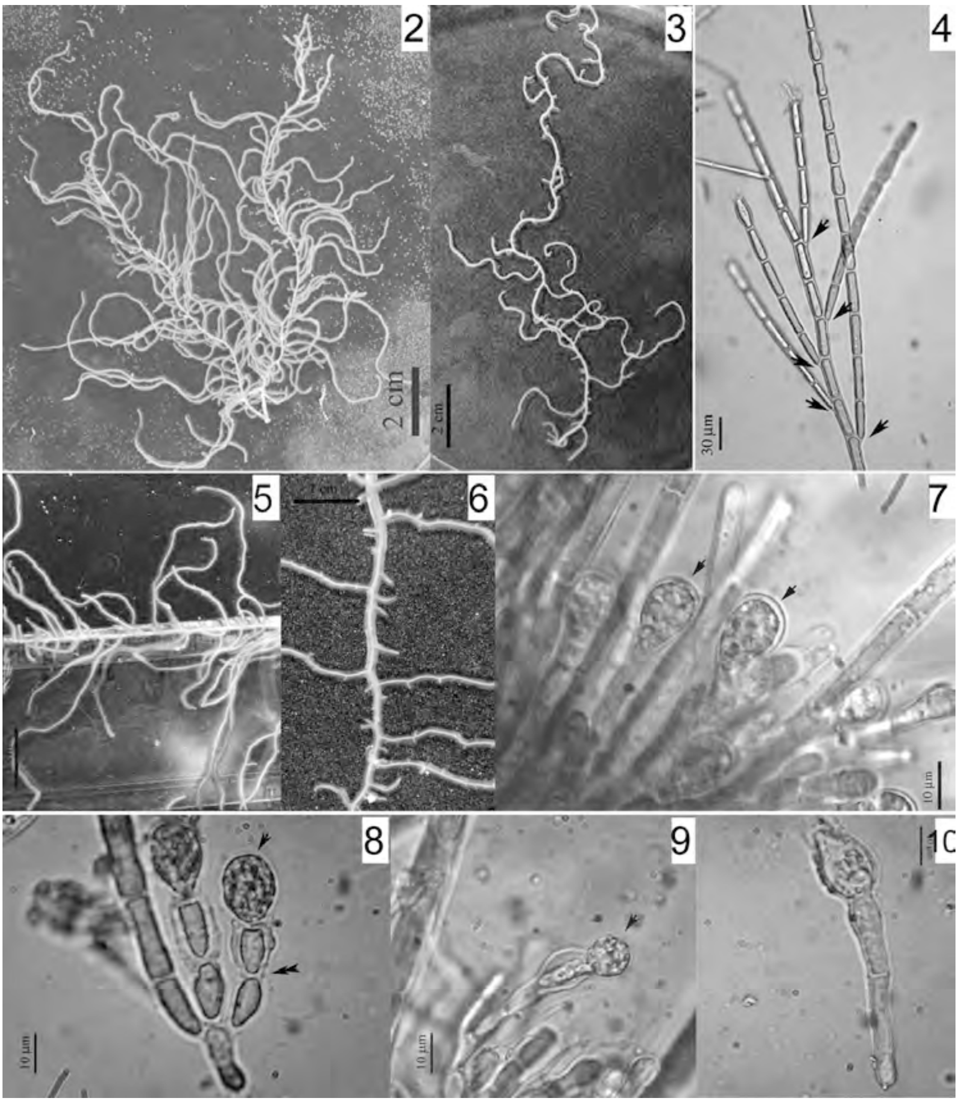
Populations	*Plant length (cm)*	*Branches per 30 mm*	*Plant diameter (µm)*	*Medulla diameter (µm)*	*Number of branch of assimilatory filaments*	*Assimilatory filaments length (µm)*	*Number of cells of assimilatory filaments*	*Cell length (µm)*	*Cell diameter (µm)*
BT1* (1996)	15 - 30	4 - 36	540 - 1 000	300 - 660	0 - 3	400 - 750	13 - 20	31.8-44	7-9.5
BT1* (2005)	18 - 50	3 - 14	600 - 1 800	400	0 - 3	470 - 700	18 - 20	33.3 – 36	6.6- 13
S1* (2008)	to 25	10 - 15	700 - 1 600	165 - 300	0 - 3 (5)	500 - 600	18 - 20	28-38	6.6 - 9
Sheath *et al*. (1993)		11- 41	513 - 1 890	88 - 611	variable	265 - 624	10 - 20	29.3-45.5	4.6 - 8.8
Carmona & Necchi (2001)	0.8 - 80	0 - 22	138.5 - 1 929.0	112.2 - 625.0	0 - 3 (4)	61.3 - 686.7	–	–	–
Kumano (2002)	100	11 - 41	513 - 1 890	88 - 611	rare	700 - 1 400	18 - 20	18-40	6 -10

* According to Material and Methods.

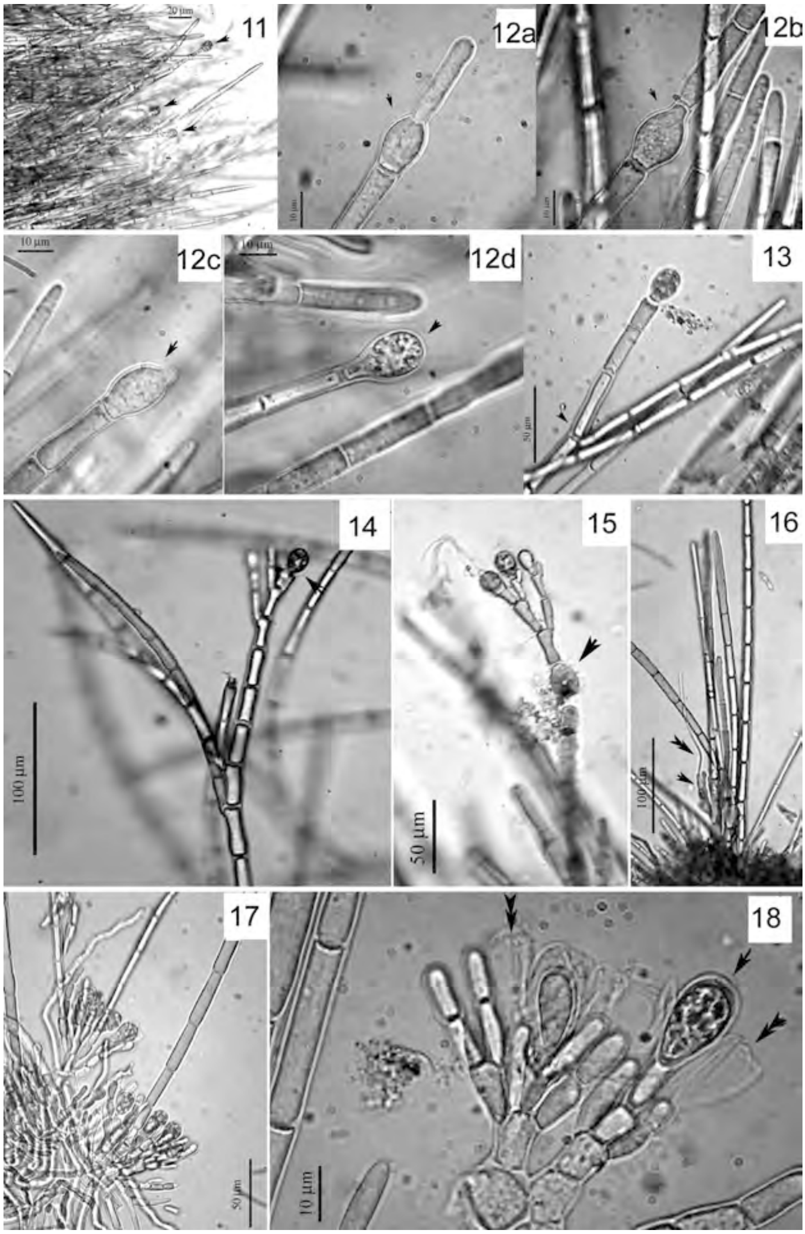
Table 3. Reproductive characteristics of *Thorea hispida* populations from Serbia compared with known characteristics of some populations of this species in the world.

		Spermatangia		*Carpogonia*		*Carposporangia*		*Monosporangia at the base of long assimilatory filament*		*Monosporangia at the top of long assimilatory filaments*	
Populations		*Lenght*	*Diameter*	*Length*	*Diameter*	*Length*	*Diameter*	*Length*	*Diameter*	*Length*	*Diameter*
BT*	(1996)			*15 - 20 trichogyne 120*	*4 - 6*	*No. 5-8*		*No. 1 - 2*		*No. 1 - 2*	
						18.5 - 26	*13.2 - 16.5*	*22.2*	*14*	*18 - 22*	*11 - 15*
	(2005)							*No. 1 - 3*		*No.1 - 2*	
								18.5 - 22	*8.14 - 17*	*18 - 21*	*11 - 14*
S1*	(2008)							*No. 1 - 3*		*No. 1 - 2*	
								16 - 22	*9 - 16*	*18 - 22*	*11 - 15*
Carmona & Necchi (2001)		*No. 2*		*14 trichogyne 280*	*3 - 7*	*No. 8*		*No. 1 - 2 - 3*		–	–
		6 - 14	*3 - 10*			*8 - 18*	*7 - 12*	*10.0 - 25.8*	*7 - 18*		
Kumano (2002)								*No. 1 - 8*			
								(8.6) - 20 - 25 (30.0)			

* According to Material and Methods; No.- number of spermatangia, carposporangia and monosporangia per clusters; all measurements are in micrometres.



Figs 2-10. Morphological features of *Thorea hispida*. **2-3**. General view of plant: **2**. locality BT1, **3**. locality S1. **4**. Non-clavate assimilatory filaments with variable length and degree of ramification (arrows). **5-6**. Details of a plant with branching: **5**. locality BT1, **6**. locality S1. **7-11**. Monosporangia: **7**. Monosporangia arising from the basal cell of the assimilatory filament (arrows). **8**. Monosporangia on a 2-celled sporangia bearing branch arising from the basal cell of the assimilatory filament (arrows). **9**. A monospora out a monosporangia (arow). **10**. Monospore germination with rhizoidal filament and empty spore.



Figs 11-18. Morphological features of *Thorea hispida*. **11**. Monosporangia of the top of the long assimilatory filaments (arrows). **12**. Occurence of the monosporangia of the vegetative celss of the long assimilatory filaments. **13**. Monosporangia attached on a 2 (3) celled sporangia bearing branch arising from which starts with the central part of the long assimilatory filament (arrows). **14-15**. Distal portion of assimilatory filaments showing monosporangia terminal on short branches (arrows). **16**. A carpogonium (arrow) with a swollen base and narrow, elongate trichogyne (arrows) on a 1-celled carpogonium-bearing branch arising from the base of the assimilatory filament. **17-18**. Clusters of carposporangia (arrow) and empty carposporangia (arrows) terminal on gonimoblast filaments.

The morphological of the plants and asexual reproduction organs of the Serbian were similar to those found in populations from all around the world (Sheath *et al*., 1993; Carmona & Necchi, 2001; Kumano, 2002) (Tables 2, 3). However, the formation of monosporangia from vegetative cells of long assimilatory filaments and their position on the tips of long assimilatory filament, or relatively long lateral assimilatory branches, has not been described to date. The existence of monosporangia on the tips of long assimilators is a characteristic of the genus *Nemalionopsis* (Kumano, 2002). In *Nemalionopsis* monosporangia are solitary or in clusters derived terminally from assimilatory filaments, and these assimilatory filaments are shorter than in *Thorea* (60-400 µm long) (Kumano, 2002). The ratio of sporangial branches length and sterile long assimilatory filament length in *Nemalionopsis* is 0.6 or more (Sheath *et al.,* 1993). Mature monosporangia of *Nemalionopsis* are elliptical or ovoidal, as in *Thorea*, but smaller (6.5-18 µm long, 5-13 µm in diameter) (Kumano, 2002). In our material, the ratio of sporangial branches length and sterile assimilatory filament length was different (from 1 to less than 0.1). The monosporangia that we observed at long assimilatory filament were larger (length 18-22 µm, width 11-15 µm) than those described in *Nemalionopsis* (Kumano, 2002).

Carmona & Necchi (2001) confirmed the coexistence of monosporangia with sexual reproductive structures (carpogonia and spermatangia), as well as carposporangia from the female ones by their slender thalli (Table 3). Our observations agree with those findings.

Thorea hispida (as *T. ramosissima*) is included in the Red List of threatened algae in some European countries: Poland - extinct species (EX) (Sieminska, 2006), Slovakia and Bulgaria - critically endangered species (CR) (Marhold & Hindak, 1998; Temniskova *et al.,* 2008), Germany – vulnerable (3) (Ludwig & Schnittler, 1996), Ukraine – rare species whose populations are small, which currently is not categorized as “endangered” or “vulnerable”, although it is in danger (III) (Шеляг-Сосонко, 1996). In Serbia, based on its distribution, population densities and exposure to anthropically-induced risk factors – such as the human exploitation of sand and gravel in the immediate proximity of the known stations for this species – *Thorea hispida* has been defined as critically endangered (CR) in this country (Simić *et al*., 2007).

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