

## Genus *Humidophila* from caves in Serbia with an improved detailed description of rare *H. brekkaensoides*

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**Abstract:** Phototrophic microorganisms can be found in biofilms at entrances to caves where they have access to sufficient sunlight, water and nutrients; however, they can also be found inside caves, where they are carried by animals and people, and can proliferate in the presence of artificial light. Although the genus *Humidophila* includes some well-known cosmopolitan species, further exploration of its rare, insufficiently investigated representatives in different geographical areas and unusual habitats is necessary. Caves remain unknown, little-explored habitats, and many species currently known to science were recorded for the first time. This study aimed to report species belonging to the genus *Humidophila* that were identified in 19 caves in Serbia. A total of ten species of this genus were recorded and some of them are documented for the first time in Serbia. The most abundant species were *H. contenta* (found in 16 caves), *H. paracontenta*, and *H. aerophila* (found in 13 caves), whereas *H. pyrenaica* was identified in only one cave. The rare species, *H. brekkaensoides* and *H. vidalii*, were also documented and described, but with different dimensions from those provided in current identification keys.

**Keywords:** biofilm; cave; diatoms; *Humidophila*; *Humidophila brekkaensoides*

### INTRODUCTION

Cave environments are generally considered to be extreme habitats, characterized by special environmental conditions and inhabited by small numbers of specialized organisms [1]. One of the specific characteristics of caves is oligotrophy, which is often a primary limiting factor for the presence of organisms in these habitats. However, the presence of various microorganisms, including phototrophic ones, has been recognized in caves [1]. Phototrophic microorganisms, aerophytic cyanobacteria and algae, are often found in biofilms near cave entrance, and have also been identified inside caves, where their appearance is the result of artificial light installations and the presence of tourists, forming a microorganism community called lampenflora [2-5]. The algal components of communities generally include many diatom representatives. Unlike freshwater diatoms,

which have been better studied in Serbia [6-9], data regarding aerophytic species remain scarce.

Diatoms have been studied in different hypogenic environments worldwide, such as in caves [2,3,10-18]. The most common diatoms found on rock substrata belong to the genera *Humidophila* [19-23], *Luticola* [23], *Orthoseira* [24, 25], *Pinnularia* [26-28] and *Hantzschia* [29]. The genus *Humidophila* (Grunow) Lowe, Kocielek, Johansen, Van de Vijver, Lange-Bertalot, and Kopalová occupy habitats such as wet rocks and areas inside of caves in the vicinity of artificial light [30].

This study aimed to investigate the distribution of species belonging to the aerophytic and cosmopolitan genus *Humidophila* in caves in Serbia, and to provide detailed morphological descriptions of these species, using light microscopy (LM) and scanning electron microscopy (SEM). Some species such as *H. brekkaensoides*

(Bock) Lowe, Kociolek, J.R. Johansen, Van de Vijver, Lange-Bertalot and Kopalová are very rare and have been insufficiently described in the literature. Therefore, one of the aims of this paper was to provide a detailed description of *H. brekkaensoides* using SEM.

## MATERIALS AND METHODS

### Study area

The caves (Supplementary Table S1) from which the samples were taken are located in eastern and western Serbia, except for Risovača Cave, which is located in central Serbia, near the city of Arandjelovac. Many of these caves are found in limestone regions of east and west Serbia are rich in cave jewelry, stalactites and stalagmites; also, rare cave roses and helictites can be found in Cerjanska Cave. Paleolithic and Neolithic human remains, as well as fossil fauna, have also been discovered in Risovača, Lazareva, Prekonoška and Petnička caves [31, 32]. The oldest explored cave in Serbia is Resavska Cave, which is approximately 80 million years old, and the oldest speleothems are approximately 45 million years old [32]. Resavska Cave contains differently colored formations that depend on the types of minerals found in the water that flows through them, including yellow from clay, red from iron-oxide and white from crystallized calcium. The longest explored cave is Lazareva Cave, which is 10000 m in length; however, the section that is open to tourists is only 900 m long [33].

### Sample collection

Diatom samples were collected by scraping biofilms from stone substrates found in 19 caves in Serbia (Supplementary Table S1), from the entrance and/or inside the caves, near artificial lights. Sampling was conducted three times a year in Resavska, Stopić, Podpeč, Lazareva, Rajkova and Risovača caves in May, July and October 2017, when changes in the diversity of phototrophic microorganisms during the tourist season were also observed. In Samar and Jezava caves, samples were taken four times a year (December, March, May, and August), even though these caves are not active tourist destinations. In other caves, samples were taken once a year from 2014 to 2017. Three to eight sampling sites were chosen, depending on the locality and presence of the biofilm. At each sampling site (located on a wall,

ceiling, or sediment) biofilms were sampled using a sterile scalpel, placed into a sterile bag, and transported to the laboratory.

### Sample preparation

All transported samples were treated with a supersaturated  $\text{KMnO}_4$  solution and concentrated HCl before rinsing with distilled water to pH 6-7 [34]. The clean diatom material was placed on microscope slides using Naphrax®.

### Sample analysis

Slide observations were performed with a Zeiss Axio-Imager M.1 light microscope using Axio Vision Release 4.9 software. The identification of genus *Humidophila* was conducted using the following literature: [20, 27, 28, 35-39]. SEM was performed using a Cambridge S4 Stereoscan (Cambridge Instruments Ltd, Cambridge, UK) at the Friedrich Hustedt Study Centre for Diatoms (BRM) in Bremerhaven, and a Zeiss Gemini Ultra plus at the Natural History Museum, London, UK.

## RESULTS

A total of ten *Humidophila* taxa were identified in Serbian caves (Table 1). Seven of these were identified in Serbia for the first time, including *H. aerophila*, *H. brekkaensoides*, *H. comperei*, *H. contemnata*, *H. gallica*, *H. pyrenaica* and *H. vidalii*, whereas the other species were recorded at other localities in Serbia (lakes in Sara Mountain; the Sava, Nišava, Rasina and Velika Morava rivers; Lepenac, Jasika and Konjski streams and Sava Lake [40-49]). The most common species were *H. contenta* and *H. paracontenta*, which were found at both the entrance and inside the caves in high numbers. *H. aerophila* was also dominant in samples taken from entrances to caves, whereas it was detected at only one site inside the cave. *H. pyrenaica*, *H. vidalii* and *H. gallica* were rarely encountered. Based on our results, descriptions of all recorded species are provided below.

***Humidophila aerophila*** (Krasske) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1, AI-AR; Fig. 6FF)

Basionym: *Navicula aerophila* Krasske

**Table 1.** Species of genus *Humidophila* in caves of Serbia.

	R	HP	D	RC	V	SV	L	BG	M	S	J	C	P	PE	RE	RI	ST	PO	RA
* <i>Humidophila aerophila</i>		*			*		*	*	*	*	*	*	*		*	*	*	*	
* <i>Humidophila brekkaensoides</i>							*		*	*		*	*		*		*		
* <i>Humidophila comperei</i>					*					*			*						
* <i>Humidophila contemnata</i>	*						*			*	*		*		*			*	*
<i>Humidophila contenta</i>		*	*	*	*		*	*	*	*	*	*	*		*	*	*	*	*
* <i>Humidophila gallica</i>							*							*					
<i>Humidophila paracontenta</i>	*			*	*		*		*	*	*	*			*	*	*	*	*
<i>Humidophila perpusilla</i>				*						*	*	*			*				
* <i>Humidophila pyrenaica</i>										*									
* <i>Humidophila vidalii</i>							*								*			*	

R – Ribnička, HP – Hadži Prodanova, D – Degurić, RC – Rćanska, V – Vernjikica, SV – Pećina kod Sove, L – Lazareva, BG – Bogovinska, M – Mandina, S – Samar, J – Jezava, C – Cerjanska, P – Prekonoška, PE – Petnička, RE – Resavska, RI – Risovača, ST – Stopić, PO – Potpeć, RA – Rajkova. A star before names presents species found for the first time in Serbia.

**Morphological characteristics (LM):** The valves are linear-elliptic with concave margins in the middle and broadly rounded ends. Length is 9.3-12.2  $\mu\text{m}$ , breadth is 2.9-3.8  $\mu\text{m}$ . The axial area is linear and broad, central zone wide. Striae are parallel, interrupted in the central area, 28-29/10  $\mu\text{m}$  (Fig. 1, AI-AR).

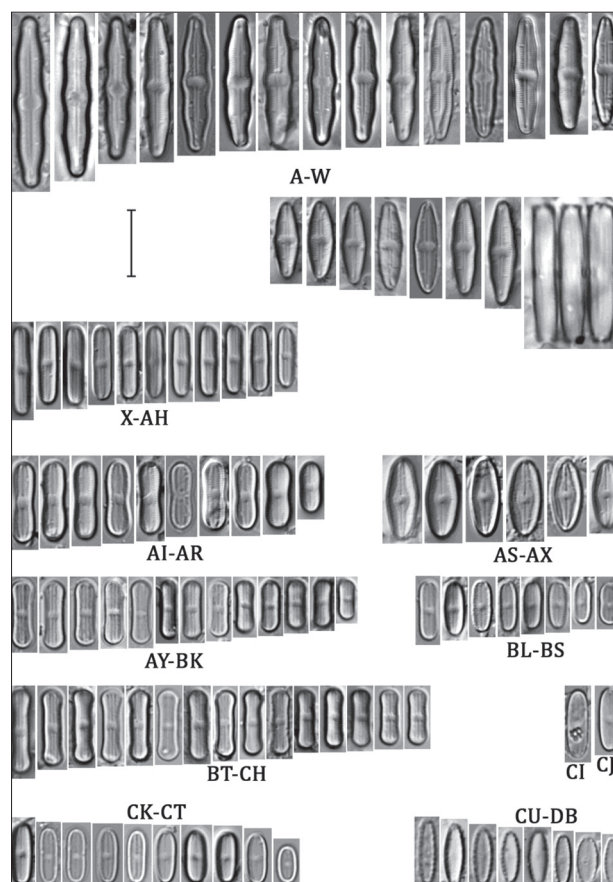
**Morphological characteristics (SEM):** The external raphe branches are straight with simple proximal endings. The axial area is a broad, central zone wide and surrounded by areolae. Areolae are slightly transversally elongated (Fig. 6F).

**Distribution in Serbia:** This species was documented in 13 caves, primarily at entrances. Only one individual was found at the sampling site inside Podpeć Cave, near the artificial light (Table 1).

***Humidophila brekkaensoides* (Bock) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1A-W; Fig. 2.A-G)**

**Basionym:** *Navicula brekkaensoides* Bock

**Morphological characteristics (LM):** The valves are linear, with protected and rounded ends. The margins are triundulate. Length is 11.6-24  $\mu\text{m}$ , breadth is 3.6-5  $\mu\text{m}$ . The axial area is linear, and narrow, with a large central area, that is round or elliptical. Striae radiate in the middle, becoming parallel at the ends, 27-31/10  $\mu\text{m}$  (Fig. 1A-W).



**Fig. 1.** Light microscopy (LM) micrographs. A-W – *Humidophila brekkaensoides*; X-AH – *H. comperei*; AI-AR – *H. aerophila*; AS-AX – *H. perpusilla*; AY-BK – *H. contenta*; BL-BS – *H. contemnata*; BT-CH – *H. paracontenta*; CI-CJ – *H. pyrenaica*; CK-CT – *H. vidalii*; CU-DB – *H. gallica*. Scale bar is 10  $\mu\text{m}$ .

Morphological characteristics (SEM): The sternum is elevated relief-like, (Fig. 2B, C, D- F). Striae, composed of one transapically elongated areola, located in a shallow longitudinal depression (Fig. 2A-F). At the apices, striae terminate after the distal raphe endings (Fig. 2B-F). The external raphe branches are straight, with simple proximal endings (Fig. 2B-D, F, G). Distal raphe endings are elevated, terminating well before the ends (Fig. 2B-F). Internal proximal raphe endings are straight, and weakly T-shaped (Fig. 2G). Distal raphe endings are straight (Fig. 2G). On the girdle, three open copulae are visible, perforated by a single row of small, transapically elongated areola (Fig. 2A).

Distribution in Serbia: This species was found sporadically at the cave entrance and was rare. However, inside Resavska Cave, the species was more abundant at both sampling points than in other caves (Table 1).

***Humidophila comperei*** (Le Cohu & Van de Vijver) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1X-AH, Fig. 3A-F)

Basionym: *Diadesmis comperei* Le Cohu & Van de Vijver

Morphological characteristics (LM): The valves are linear with broadly rounded ends. Length is 9.1-13.3  $\mu\text{m}$ , breadth is 2.2-3.1  $\mu\text{m}$ . The axial area is linear, and the central area is large. Raphe is straight, filiform. Striae are parallel, 31-36/10  $\mu\text{m}$  (Fig. 1X-AH).

Morphological characteristics (SEM): The external raphe branches are straight. The central area is round and surrounded by areolae. Areolae are transapically elongated (Fig. 3A-E). Girdle is composed of several open, perforated bands (Fig. 3F).

Distribution in Serbia: This species was found at the entrances of three caves (Table 1).

***Humidophila contemnata*** (E.Reichardt) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1BL-BS; Fig. 6D, E)

Basionym: *Diadesmis contemnata* Reichardt

Morphological characteristics (LM): The valves are linear to linear-elliptical, with broadly rounded ends. The margins are convex or slightly concave. Length is 6.2-10.4  $\mu\text{m}$ , breadth is 2.7-4.2  $\mu\text{m}$ . The axial area is

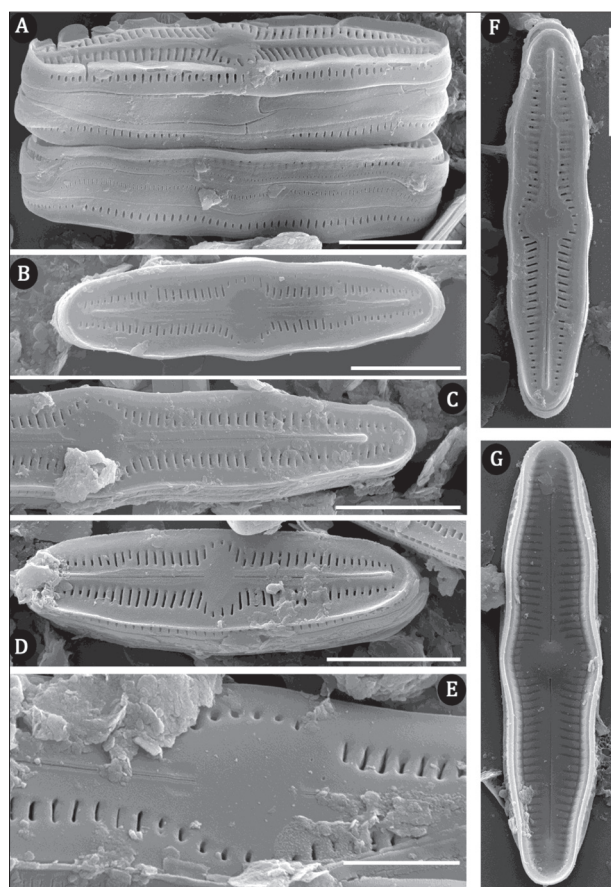


Fig. 2. SEM micrographs of *H. brekkaenoides*. Scale bar is 5  $\mu\text{m}$  in A-D, F, G, and 2  $\mu\text{m}$  in E.

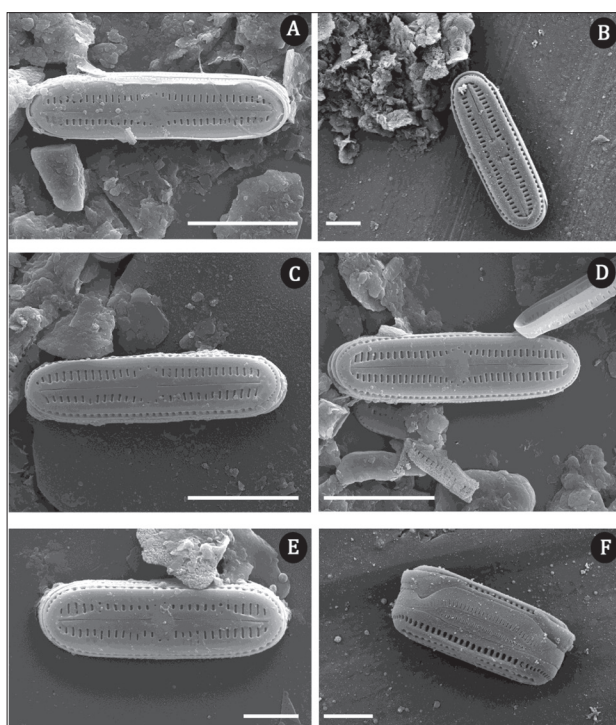
linear, the central area is small, rounded or elliptical, striae are parallel, 29-30/10  $\mu\text{m}$  (Fig. 1BL-BS).

Morphological characteristics (SEM): The axial area is broad (Fig. 6D, E). The external raphe branches are straight, with T-shaped distal raphe endings (Fig. 6D). The areolae are slightly transapically elongated (Fig. 6D, E).

Distribution in Serbia: *H. contemnata* was recorded in eight caves, primarily at the entrances. In addition, the species was found individually inside the Resavska and Rajkova caves, on the ground, and inside Lazar Cave, on the stone wall (Table 1).

***Humidophila contenta*** (Grunow) Lowe, Kociolek, J.R. Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1AY-BK; Fig. 4A-F)

Basionym: *Navicula contenta* Grunow



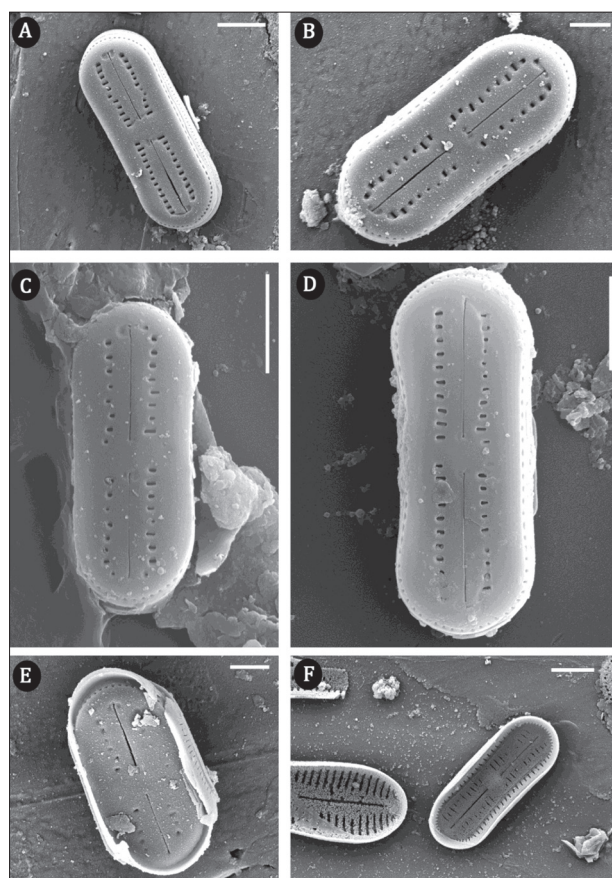
**Fig. 3.** SEM micrographs of *H. comperei*. Scale bar is 5 µm in A, C, D, and 2 µm in B, E, F.

**Morphological characteristics (LM):** The valves are linear, with broadly rounded ends and slightly concave margins. Length is 3.7-11.6 µm, breadth is 1.9-3.4 µm. Striae are parallel and interrupted in the center and difficult to resolve by LM (Fig. 1AY-BK).

**Morphological characteristics (SEM):** Central fascia is present (Fig. 4A-E). The axial area is wide and linear (Fig. 4A-E). The internal raphe branches are straight (Fig. 4F). The areolae are slightly transapically elongated, 40-50/10 µm (Fig. 4A-E).

**Distribution in Serbia:** This species is found in most caves, except the Rćanska, Petnička, and Pećina kod Sove caves. It was found at different sampling sites (outside and inside of caves, on wet and dry walls, as well as on the ground and near artificial light) (Table 1). *H. contenta* was previously recorded in different lakes in Sara Mountain [40-42], Lepenac spring [43], Temska River [44], Zapadna Morava River [45], Dojkinci River [46], Sava Lake [47] and the Velika Morava and Sava rivers [48].

***Humidophila gallica*** (W.Smith) Lowe, Kociolek, Q. You, Q. Wang & Stepanek (Fig. 1, CU-DB)



**Fig. 4.** SEM micrographs of *H. contenta*. Scale bar is 2 µm in A, C, D, F, and 1 µm in B, E.

**Basionym:** *Diadesmis gallica* W. Smith

**Morphological characteristics (LM):** The valves are linear-elliptical, with broadly rounded ends. Length is 7.7-9.3 µm, breadth is 2.7-3.5 µm. Marginal spines are well developed, whereas valve interiors are not clearly visible in LM. Striae are not visible in LM (Fig. 1CU-DB).

**Distribution in Serbia:** This species was recorded on wet soils inside two caves (Table 1).

***Humidophila paracontenta*** (Lange-Bertalot & Werum) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014: 358 (Fig. 1BT-CH; Fig. 5, A-F)

**Basionym:** *Didaesmis paracontenta* Lange-Bertalot & Werum

**Morphological characteristics (LM):** The valves are parallel with broadly rounded sub-capitate apices. Length is 7.7-11.4 µm, and breadth is 2-3.4 µm. The

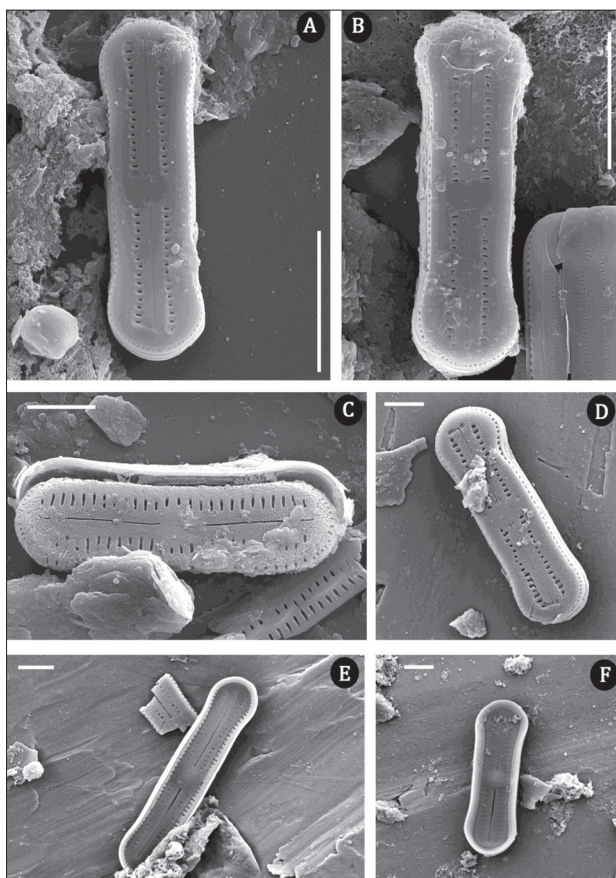


Fig. 5. SEM micrographs of *H. paracontenta*. Scale bar is 5 µm in A, B, and 2 µm in C-F.

axial area is narrow, and linear, whereas the central area is broad and rounded. Striae are fine, parallel, difficult to resolve by LM (Fig. 1, BT-CH).

Morphological characteristics (SEM): Central fascia is present (Fig. 5A-D), and sometimes surrounded by areolae (Fig. 5C). The external raphe branches are straight (Fig. 5A-D), sometimes with T-shaped distal raphe endings (Fig. 5D). The internal raphe branches are straight (Fig. 5E, F). The areolae are transapically elongated, approximately 40/10 µm (Fig. 5A-D).

Distribution in Serbia: This species was recorded in 13 caves (at entrances and on walls and soil inside caves) (Table 1). In addition, *H. paracontenta* was previously recorded in the Rasina River and Jasika and Konjski streams [49].

***Humidophila perpusilla*** (Grunow) Lowe, Koci-olek, J.R. Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1AS-AX; Fig. 6B, C)

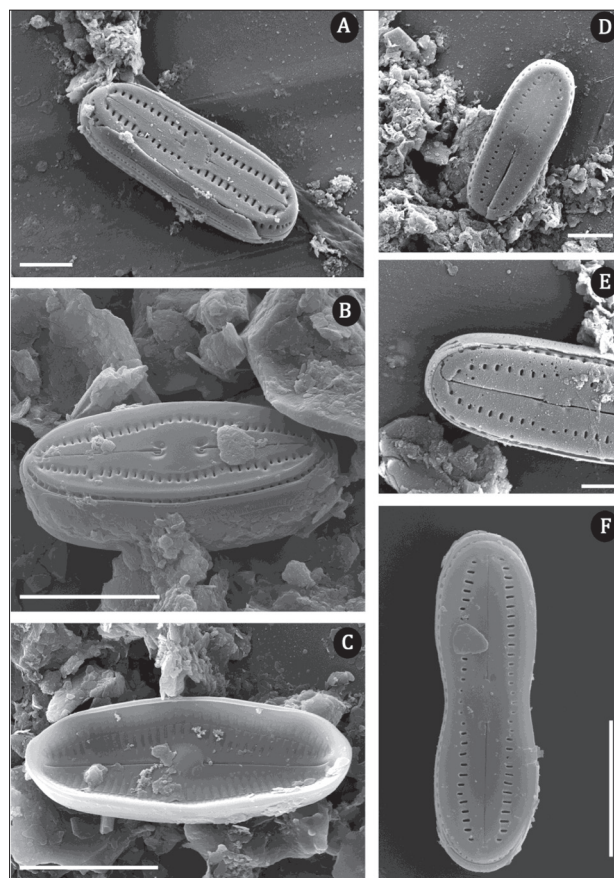


Fig. 6. SEM micrographs of A– *H. pyrenaica*; B, C– *H. perpusilla*; D, E– *H. contemnata*; F– *H. aerophila*. Scale bar is 5 µm in B, C, F, and 2 µm in A, D, E.

Basionym: *Navicula perpusilla* (Grunow) D.G.Mann

Morphological characteristics (LM): The valves are elliptic-lanceolate to linear-lanceolate, with rostrate to broadly rounded ends. Length is 9.2-14 µm, and breadth is 3.8-5.2 µm. The axial area is wide with a large central area. The raphe appears filiform, and linear. The striae are radiate, 25-30/10 µm (Fig. 1, AS-AX).

Morphological characteristics (SEM): The external and internal raphe branches are straight (Fig. 6B, C). The areolae are transapically elongated (Fig. 6B).

Distribution in Serbia: This species was found in five caves, mainly at entrances to caves. Among the lampenflora community, this species was only found in Resavska Cave, on the wet walls and soils (Table 1). *H. perpusilla* has previously been recorded in different lakes of the Shara Mountain [40-42] and the Dojkinci River [46].

***Humidophila pyrenaica*** (Lange-Bertalot & Werum) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová (Fig. 1CI, CJ; Fig. 6A)

Basionym: *Diademesmis pyrenaica* Lange-Bertalot & Werum

Morphological characteristics (LM): The valves are linear with broadly rounded ends. Length is 9.1-10µm, and breadth is 2.8-3.1µm. The raphe is straight, and filiform, and the axial area is broad, with a slightly wide central area. The striae are parallel and difficult to resolve by LM (Fig. 1CI, CJ).

Morphological characteristics (SEM): The external raphe branches are straight. The central area is round and surrounded by areolae. Areolae are transapically elongated, approximately 35/10 µm (Fig. 6A).

Distribution in Serbia: *H. pyrenaica* was found in samples taken from Samar Cave and was registered in only one season (May) (Table 1).

***Humidophila vidalii*** (Van de Vijver, Ledeganck & Beyens) Lowe et al. (Fig. 1,CK-CT)

Basionym: *Diademesmis vidalii* B. Van de Vijver & P.Ledeganck

Morphological characteristics (LM): The valves are linear, with broadly rounded ends. Length is 5.8-10.4 µm and breadth is 2.6-3.7 µm. The axial area is wide, linear, and the central area is absent. The raphae are straight, and filiform. Striae are not visible in LM (Fig. 1CK-CT).

Distribution in Serbia: This species was found in three caves. This species was most abundant at the entrance of the Resavska Cave, where samples were taken from the walls, but it was also found sporadically inside the Lazareva and Podpeč caves (Table 1).

## DISCUSSION

The genus *Humidophila* contains well-known diatoms that are usually found on wet rocks, walls, wet soils, moss vegetation and in cave habitats [39, 50]. The genus includes diverse species, which have been described in Europe [38, 50], China [30], Hawaii [21] and the Antarctic region [22]. The taxa in this genus possess a small, linear to lanceolate-elliptic valve and broadly

rounded ends. Most of the species were originally classified as belonging to the genus *Diademesmis* [51], subgenus *Paradiademesmis* (Lange-Bertalot & Le Cohu), but after detailed morphological examinations, they were transferred into the genus *Humidophila* [21].

Phototrophic microorganisms are expected to be found on the walls and rocks at entrances of caves because they can receive sufficient natural light, water (from precipitation, dew or the nearest river) and nutrients in these locations [1, 52]. They can also be carried inside the cave by air, animals, water and humans, and in the presence of artificial light, they can colonize different substrates such as walls, ceilings, and cave jewelry [2, 4]. Our results show ten species recorded in 19 caves in Serbia. Some of these species, such as *H. contenta*, *H. paracontenta* and *H. perpusilla*, have previously been documented in Serbia in different habitats, whereas the other species were recorded in the Serbia for the first time. *H. contenta* was previously recorded in different lakes of the Shara Mountain [40-42], Lepenac spring [43], Temska River [44], Zapadna Morava River [45], Dojkinci River [46], Sava Lake [47], Velika Morava and Sava rivers [48] with a large water-temperature range [39]. In addition to the caves, *H. paracontenta* has been found in Rasina River and Jasika and Konjski streams [49], whereas *H. perpusilla* has been documented in different glacial lakes of the Shara Mountain (e.g. Veliko Jažinačko, Malo Jažinačko, Mali Vir I, Mali Vir II) [40-42] and the Dojkinci River [46]. As was expected, *H. aerophila*, *H. contenta*, *H. contemnata* and *H. paracontenta* were mostly found at the entrances to the caves, as these species are typical terrestrial diatoms that can be found on the wet rocks, soil and different cave substrata [21,52]. These species were also sporadically found inside caves, near artificial light, except *H. contenta* and *H. paracontenta*, which were found inside the caves in high abundance. As a cosmopolitan species, in the presence of adequate conditions, they can easily colonize substrates, such as the wet soil and wet walls found in caves. Although cosmopolitan species tend to be very common on wet rocks and walls [39], *H. gallica* was found at low abundance inside only two caves (Lazareva and Resavska) on wet soil, whereas *H. pyrenaica* was registered in only one cave entrance, but at a low abundance. High relative humidity (RH) and artificial light favored *H. gallica* growth [39]; however, because this species is very rare in biofilms, caves may not represent the primary

habitat of this species and it may not be fully adapted to these conditions. *H. gallica* likely entered the caves via air, animals, or tourists, like *H. aerophila*, which was detected at the entrances of caves and in only one other location inside Podpeč Cave. This species is typically aerophytic and likely entered the cave via air flow and found suitable conditions for development due to the presence of artificial light. However, only one *H. aerophila* individual was recorded, so that it is difficult to determine whether it is capable of colonizing the substrate in higher numbers. *H. comperei* was recorded at the entrances of three caves (Vernjickica, Samar, Prekonoška). According to Cohu and Van de Vijver [53], this species was found at Île de la Possession (Crozet Archipelago) on dry soil, whereas our results show that it can be found on cave walls where the RH is high (Samar Cave, found in March, May and December). This is a rarely described species that most likely has a wide range of habitats. *H. pyrenaica* was only detected at the entrance of Samar Cave in May, although samples were taken four times during a year. Unlike the inside of caves, where environmental parameters are generally stable (temperature (T) and relative humidity (RH)), these parameters are much more variable at the entrances to caves. Generally, T and RH vary, not only by season, but also between the day and night [1], which can result in changes in the phototrophic communities. According to Asencio and Aboal [54], during a two-year study, T and RH varied significantly during the day and throughout the year, T values ranged from 1.6 °C to 39.1 °C, whereas RH varied up to 77 %. The type of substrate and its heat capacity have also been suggested to affect the composition of phototrophic communities [1]. Inside caves, phototrophic communities will grow near natural light, unless artificial lights have been installed in the caves, allowing phototrophic communities to develop deeper within the cave environment [55,56].

The dimensions and descriptions of the recorded species in this study correspond with the descriptions from the identification keys, except for the rarely found species *H. brekkaensoides*, which was first described by Bock [57], who discovered it in a thin biofilm layer on rocks in the Alps. According to Bock's findings [57], *H. brekkaensoides*, (*Navicula brekkaensoides*) can not be found on limestone because it prefers non-calcareous substrates. However, this species appears to occur in a wider spectrum of habitats, as the results of Buczkó [20]

and this study have shown that *H. brekkaensoides* can be found on limestone substrates. In addition, Reichardt [37] recorded this taxon in the Hochlantsch Mountain in Austria, from a slightly shaded limestone depression, and therefore whether this species prefers calcareous or non-calcareous substrates remains unclear as further details about the habitats are missing. Unlike cave entrances where *H. brekkaensoides* was sporadically found, the population of this species was more abundant inside the cave near artificial light, which is likely the result of more stable environmental conditions (especially T and RH) and a tolerance for low nutrient input [52]. Furthermore, the dimensions of the valves reported by Bock [57] are different from those observed in our study. According to Bock [57], valve length is 17-23 µm and the breadth is 4-5 µm, whereas in our population, the length is 11.6-24 µm and the breadth is 3.6-5 µm. Compared with the description by Chattova et al. [39], the dimensions of *H. vidalii* were also different in our study. Chattova et al. reported a length of 7-17.5 µm and a breadth of 2-3.5 µm, whereas in our population the length is 5.8-10.4 µm and the breadth is 2.6-3.7 µm.

*Humidophila* species in rivers and streams are usually recorded in the middle and lower reaches where T is higher, but this species has also been found in glacial lakes where T is lower [44,46]. They are widespread species with mechanisms that allow them to adapt to different environmental conditions and different types of substrates [1,52]. For example, *H. contenta* was recorded in the Nišava, Temska and Dojkinci rivers where T varies from 0.1-27 °C for the Nišava River, from 10-18 °C for the Temska River, and from 6.5-12.5 °C for the Dojkinci River [44, 46]. The same species were found in different habitats that were characterized by a wide range of T, RH, and pH values (8-14 °C, 65-100 %, pH=3.7 and 6, respectively) [13, 58]. Probably, all cosmopolitan species are likely able to easily adapt to rapid changes in environmental conditions. Unlike freshwater habitats or cave entrances where environmental parameters are variable and dependent on the external climate, these parameters are stable throughout the year inside of caves. Changes in these parameters may be observed if the cave entrances are very large, enabling the outside climate to influence the fragile and stable condition inside the cave [1,19], or due to the installation of artificial light, which influences the primary factors necessary for the growth of phototrophs, including: providing light, increasing T and decreasing RH.



Data regarding the morphological and physiological characteristics of diatoms found in caves remain poorly described. According to Falasco [52], some species can exhibit morphological modifications when they grow in different environmental conditions. Generally, diatoms from caves display smaller sizes and high resistance to desiccation, which are likely adaptations to life in extreme habitats with low nutrient input [52]. Because caves are described as oligotrophic habitats, with low nutrient input, low T, high RH, and low light [59] compared with the outside environment, species in caves compete for the same resources, which could affect the valve size.

Data regarding diatom diversity in caves remain scarce in the literature. Although these species are known to science, they have been identified for the first time in the caves of Serbia, and it is likely that the list of species present in these environments is incomplete. Moreover, details regarding morphological and physiological characteristics of these diatoms are still lacking, necessitating further research to obtain a better understanding of these communities.

## CONCLUSIONS

These results describe species of the genus *Humidophila* recorded in the caves of Serbia. Of the ten species identified; seven were found for the first time in Serbia, whereas the others have been registered in different habitats in Serbia (lakes, rivers, and streams). They were found at the entrance and inside tourist caves, near artificial light. The study includes a description of the rare species *H. brekkaenoides*, with a different range of valve dimensions than in the first reported description. Because the caves of the Balkan Peninsula have not been sufficiently explored in terms of their phototrophic microorganisms, it is necessary to conduct further research to obtain more information regarding this and other diatom species.

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### Supplementary Material

The Supplementary Material is available at: [http://serbiosoc.org.rs/NewUploads/Uploads/Nikolic%20et%20al\\_5125\\_Supplementary%20Table%20S1.pdf](http://serbiosoc.org.rs/NewUploads/Uploads/Nikolic%20et%20al_5125_Supplementary%20Table%20S1.pdf)