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NEW FINDS OF NAKED AMOEBAE (PROTISTA) IN WATER RESERVOIRS OF UKRAINE

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New Finds of Naked Amoebae in Water Reservoirs of Ukraine. Patsyuk, M. K. — In the water bodies of Ukraine, 6 new species of naked amoebae were found: *Saccamoeba* sp., *Ripella* sp., *Vannella lata* Page, 1988, *Thecamoeba* sp., *Acanthamoeba* sp., *Vahlkampfia* sp. According to the current taxonomy, they belong to 3 classes, 4 orders, 5 families and 6 genera. New localities and original descriptions of the species are presented, along with brief characteristics of the corresponding genera. *Thecamoeba* sp. and *Acanthamoeba* sp. are first reported from the territory of Ukraine.

Key words: naked amoebae, water reservoirs, Ukraine.

Introduction

In our previous surveys, 45 species of naked amoebae were found in water bodies of the Ukrainian Polyssya; the findings allowed to analyze the specifics of the protists' habitats (Patsyuk, 2010, 2011 a, b, 2012 a, b, 2013 a, b, 2014 a–d, 2015 a, c; Patcyuk, Dovgal, 2012). The species found were assigned to lobose, filose and heterolobose amoebae. Previously, we also found 9 naked lobose sea-dwelling species among the amoebae of the Black Sea in Sevastopol vicinities (Patsyuk, 2015 b).

The list of the protists in the fauna of Ukraine is expanding due to the examination of different types of water reservoirs. During our faunistic studies on protists from water reservoirs of Ukraine we first found two species of amoebae (*Thecamoeba* sp., *Acanthamoeba* sp.) and recorded new localities for some previously known species (*Saccamoeba* sp., *Ripella* sp., *V. lata*, *Vahlkampfia* sp.). In the present study, most species of amoebae were identified to the generic level based on their morphological examination. The present work complements a cycle of previously published papers dealing with morphological characterization of naked amoebae from water bodies of Ukraine.

Material and methods

The field research was carried out during 2009–2015 in various water bodies of Ukraine. In the laboratory, the amoebae were cultivated in Petri dishes with non-nutrient agar as the medium after F. Page (Page, 1988; Page, Siemensma, 1991). They were observed under Zeiss Axio Imager M1 light microscope using differential interference contrast at the Centre of collective use of scientific equipment "Animalia" of the I. I. Shmalhausen Institute of Zoology, NAS of Ukraine (Kyiv). Amoebae species were identified with the help of separate publications on naked amoebae systematics: Page (1988), Page, Siemensma (1991), Smirnov, Goodkov (1993), Smirnov, Goodkov (1999), Brown, Smirnov (2004); Smirnov (2008), and Smirnov et al. (2011).

Results and discussion

In the water bodies of Ukraine we identified 6 species of naked amoebae. The new localities are shown on the fig. 1. The list of the protists arranged according to the current systematics (Smirnov et al., 2011; Bass et al., 2009) with brief morphological species essays is given below.

Fig. 1. New records of naked amoebae in water bodies of Ukraine: ● — *Saccamoeba* sp., ● — *Ripella* sp., ○ — *V. lata,* ○ — *Thecamoeba* sp., ■ — *Acanthamoeba* sp., □ — *Vahlkampfia* sp.

Class TUBULINEA Smirnov, Nassonova, Berney, Fahrni, Bolivar et Pawlowski, 2005
Order EUAMOEBIDA Lepsi, 1960
Family HARTMANNELLIDAE Volkonsky, 1931
Genus *Saccamoeba* Frenzel, 1892

Amoebae of the genus *Saccamoeba* belong to the monotactic morphotype. The shape of these protists's bodies is wormlike or clavate. At the anterior end of the cell there is a small area of hyaloplasm. The uroidal structures are of the villous-bulbous type. The nucleus is of the vesicular type, the cytoplasm contains bipyramidal crystals. Most species form cysts (Page, 1988; Patsyuk, 2012 a).

The species of the genus live in fresh and salt water, and soil (Alimov, 2000).

Saccamoeba were found in the water bodies of Europe, North and South America (Page, Siemensma, 1991).

Although we observed only one distinct species of the genus, the morphological data were not sufficient to identify it to the species level; that is why the protist is referred to as *Saccamoeba* sp. (fig. 2).

The organism belongs to the monopodial morphotype. The cells are clavate, relatively wide, the hyaline cap almost disappears in motion. The cytoplasm moves relatively fast, it forms short pseudopodia when the amoeba begins to move in another direction. The single contractile vacuole can be situated anywhere in the granuloplasm. The uroid is distinctly defined, relatively large, and belongs to the villous-bulbous type.

The length is 65–92 μm, width is 30–32 μm, the L/B ratio is 3–3.2.

The single nucleus is of the vesicular type, its diameter is around 8 μm.

We did not observe cyst formation in the culture.

Previously, we have found the species in the water bodies of Volyn Polissya (Patcyuk, Dovgal, 2012). The new locality is the Cherevakha River (Manevichi District, Volyn Region).

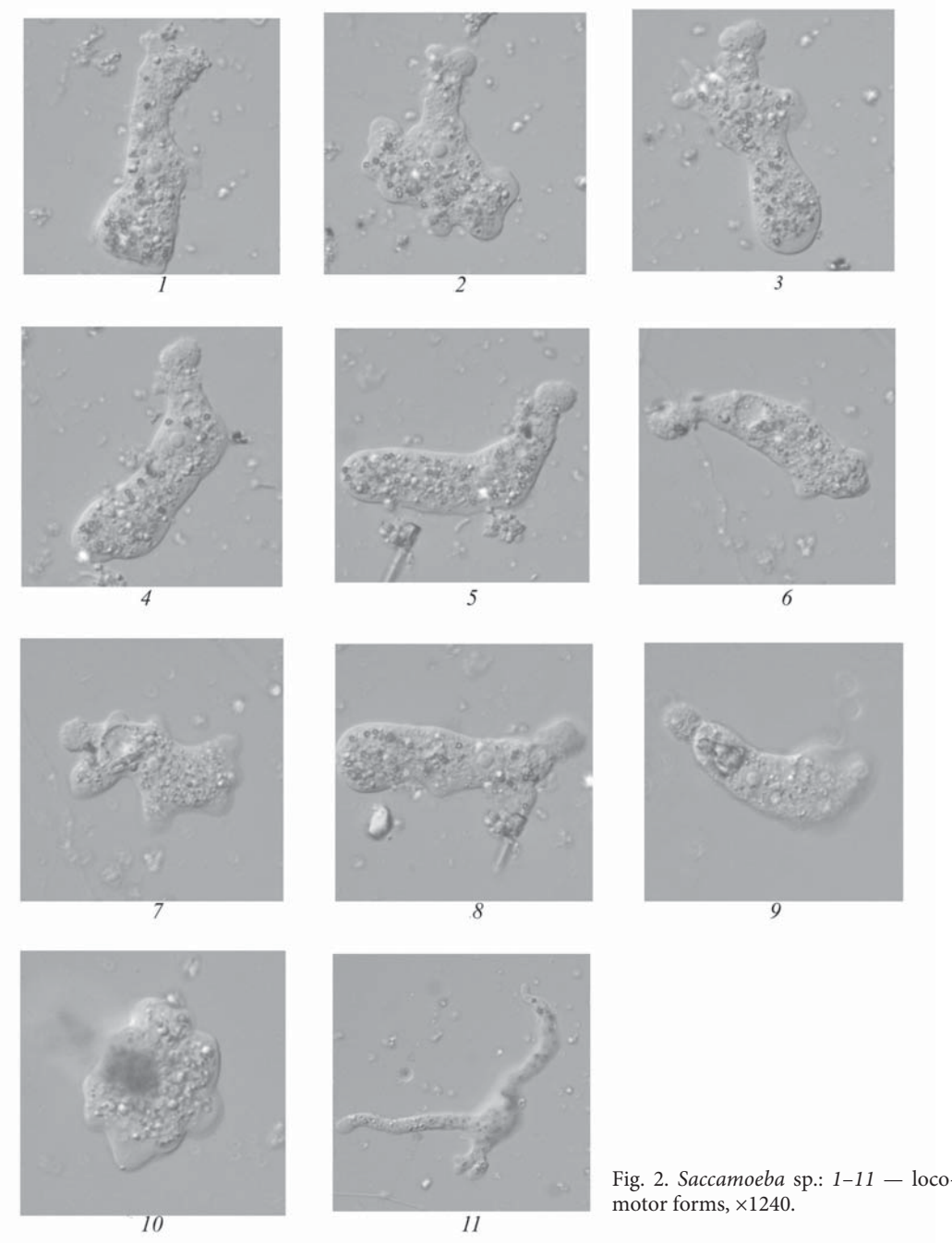

Fig. 2. *Saccamoeba* sp.: *1–11* — locomotor forms, ×1240.

Class DISCOSEA Cavalier-Smith, Chao et Oates, 2004
Order VANNELLIDA Smirnov, Nassonova, Berney, Fahrni, Bolivar et Pawlowski, 2005
Family VANNELLIDAE Bovee, 1970
Genus *Ripella* Smirnov, Nassonova, Chao et Cavalier-Smith, 2007

The amoebae belonging to the genus *Ripella* are of the fan-shaped morphotype, small, disc-like. The frontal area of the hyaloplasm is well-defined. They move relatively fast. The amoebae never form subpseudopodia and dorsal ridges. In this genus, no specific uroidal structures are seen. The cell's posterior can be smooth, conical, or elongated. The nucleus is single.

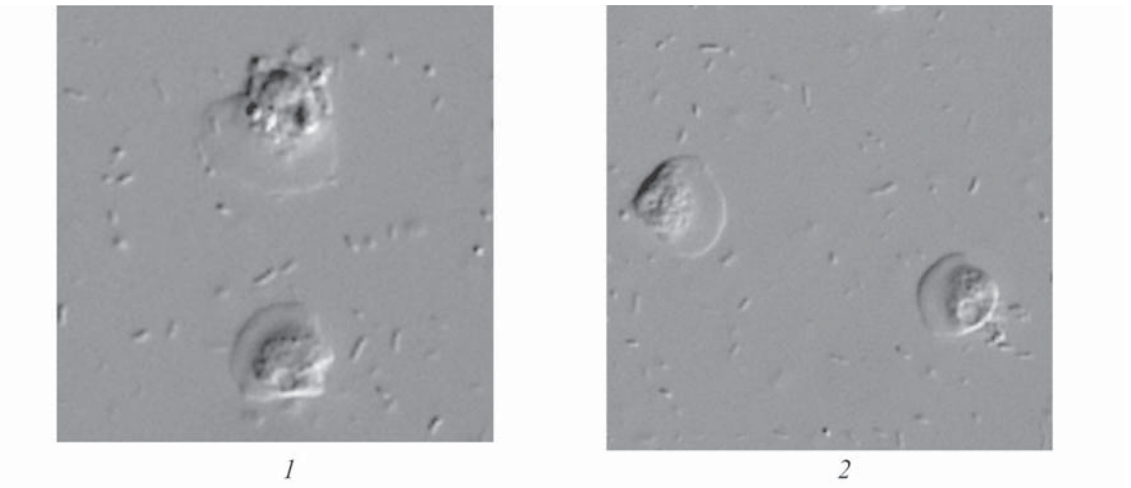

Fig. 3. *Ripella* sp.: *1–2* — locomotor forms, ×1240.

Species of the genus were found in the water bodies of Europe, North America, India (Page, Siemensma, 1991).

The amoebae we found belong to the fan-shaped morphotype (fig. 3). They are small, the posterior of the cell is a half-circle. The hyaloplasm occupies about a half of the cell's area. The posterior of the cell, where the granuloplasm is located, contains the contractile vacuole. There are no uroid structures.

The length of the cell is 8-16 μm, the width is 16–30 μ, the L/B ratio is 1.5–1.7.

The single nucleus is nearer to the frontal area of the hyaloplasm. Its diameter is 2.3–3 μm.

The floating form is of the radial type with short pseudopodia stemming from the central mass of the cytoplasm.

In the cultures, we did not observe cysts.

Previously we collected the species in the water bodies of Zhytomyr and Volyn Polissya (Patcyuk, Dovgal, 2012). Here we report the new localities: Maryanivka village (Vasilkiv District, Kyiv Region), the rivers Dnipro and Ingulets (Kherson Region).

Genus *Vannella* Bovee, 1965

The genus *Vannella* contains amoebae of the fan-shaped morphotype. When in motion, their shape is either fan-like or a half-circle, a crescent, or shovel-like. Most species have single vesicular nuclei, while some have peripheral nucleoli. They are capable of producing floating forms; this ability is one of the main diagnostic features. There were some cases of cyst formation observed.

There are species that live in fresh and salt water and in soil (Alimov, 2000).

Vannella lata Page, 1988

The species belongs to the fan-shaped morphotype (fig. 4). The locomotive forms are crescent in shape. The amoeba is wider than long. The hyaline edge elongates when the cell changes the direction of its movement and hugs the cell's sides. The cytoplasm does not form pseudopodia or subpseudopodia. There are no uroidal structures. The contractile vacuoles are mostly situated near the hyaline edge. The cell's posterior is conical. The dimensions of the locomotive form are 35–52 μm, the L/B ratio is 0.5–1.5. The single nucleus is 3–5 μm in diameter. The floating form has long sharp radial subpseudopodia (fig. 4, *8*).

We did not observe any cyst formation.

The diagnosis of the species includes a fan-shaped body, the cell's width is twice its length, the floating form has long sharp pseudopodia whose length exceeds their diameter (Page, Siemensma, 1991).

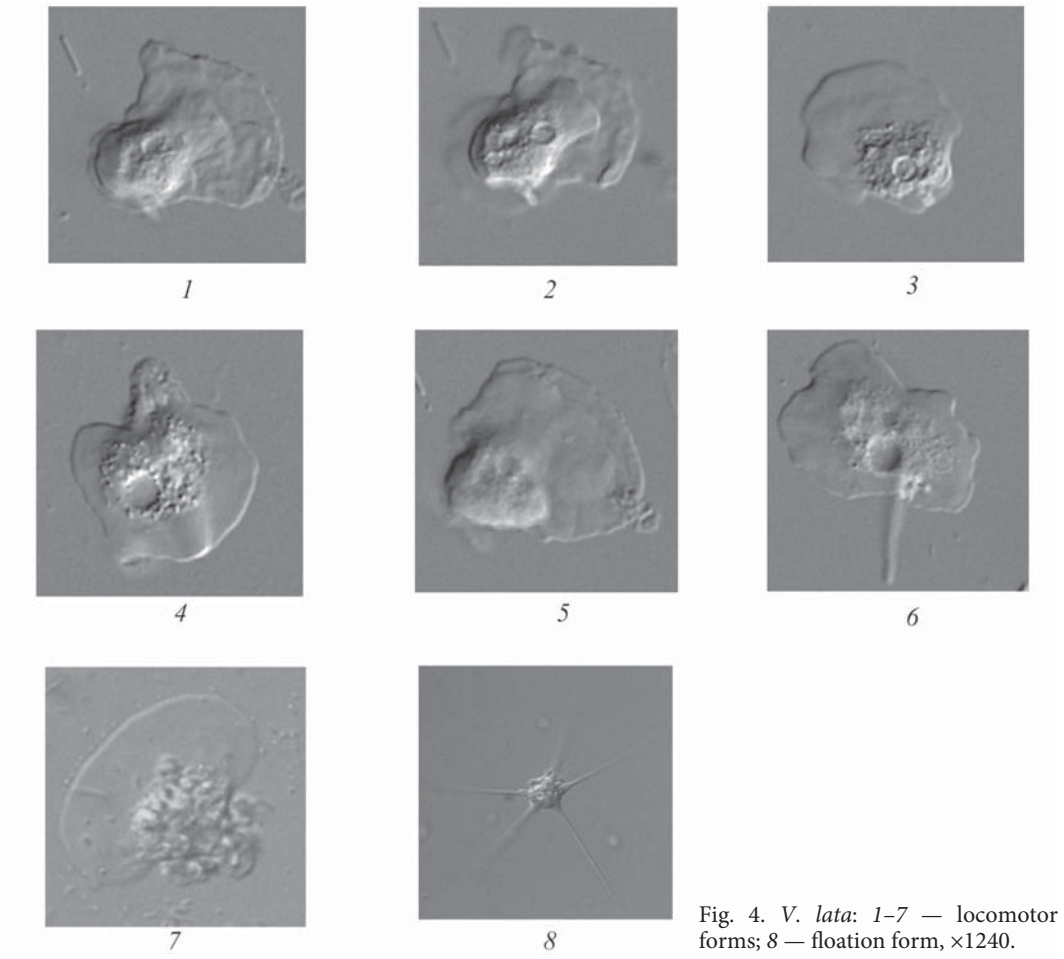

Fig. 4. *V. lata*: *1–7* — locomotor forms; *8* — floation form, ×1240.

V. lata is known from the water bodies of Europe (Page, Siemensma, 1991).

We have previously found *V. lata* in the water bodies of Zhytomyr, Volyn and Kyiv Polissia (Patcyuk, Dovgal, 2012; Patsyuk, 2014 c). The Dnieper River in the vicinities of Kherson (Kherson Region) is a new locality

Order THECAMOEBIDA Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family THECAMOEBIDAE Schaeffer, 1926
Genus *Thecamoeba* Fromentel, 1874

Species of the *Thecamoeba* do not form pseudopodia. The cell moves as a whole, it is capable to form dorsal ridges and hyaloplasm folds. These amoebae belong to the striate or rugose morphotypes. Most species of the genus lack uroidal structures; for some, bulbose or morulate uroids were described. *Thecamoeba* species have single nuclei.

Thecamoeba can live in fresh and sea water and in soil (Alimov, 2000).

The genus is known from the water bodies of Asia, Europe, North America, India (Page, Siemensma, 1991).

Flattened amoebae, oval in shape, of the striate morphotype (fig. 5). The cell has smooth edges. When in motion, the hyaloplasm forms folds and parallel dorsal ridges, and the amoeba's shape can change: an elongated, oval amoeba pulls itself to its frontal edge, the cell becomes a half-circle, the hyaloplasm edges almost the whole cell from both sides, and then the cell becomes slender again.

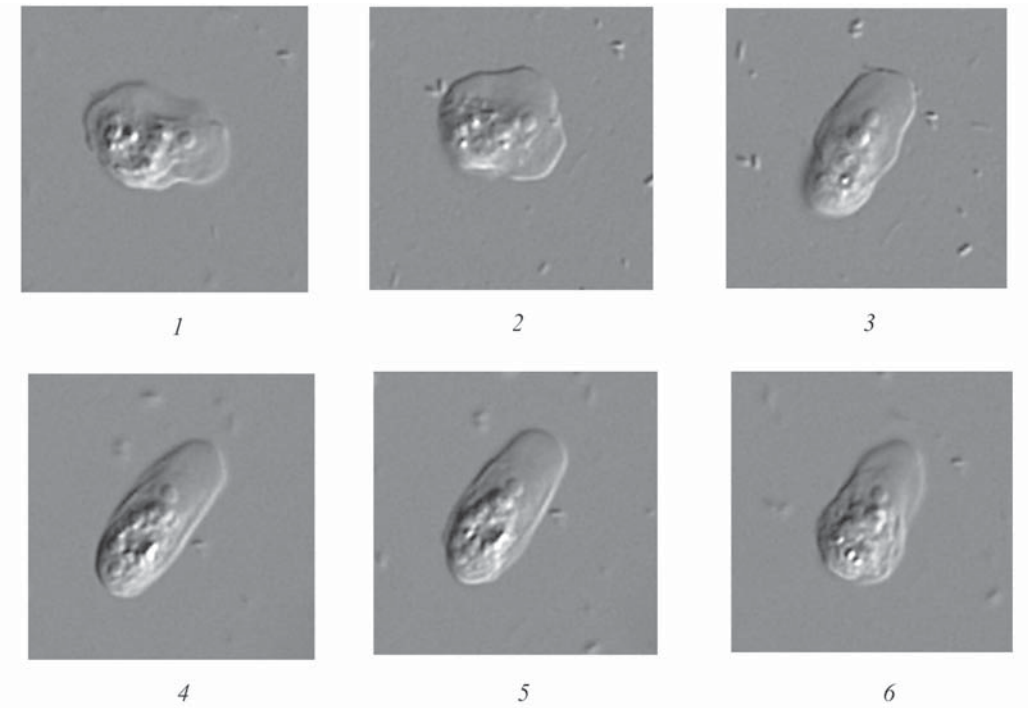

Fig. 5. *Thecamoeba* sp.: 5–*6* — locomotor forms, ×1240.

There are no differentiated uroidal structures. The single contractile vacuole is in the central region of the granuloplasm. The cell length is 25–40 μm, width is 12–18 μm, the L/B ratio is 1.5–2.5; the nucleus is vesicular, 1.5–2.5 μm in diameter.

The species is first reported from the Dnieper River in the vicinities of Kherson (Kherson Region).

Order CENTRAMOEBIDA Rogerson et Patterson, 2002
Family ACANTHAMOEBIDAE Sawyer et Griffin, 1975
Genus *Acanthamoeba* Volkonsky, 1931

The locomotive forms of all species in the genus *Acanthamoeba* belong to the acanthopodial morphotype. They are, as a rule, flattened, with sharp and short subpseudopodia. The nucleus is vesicular. They produce cysts with pores. The endocyst is often shaped like a star (Page, Siemensma, 1991).

Some *Acanthamoeba* (*A. castelanii, A. culbertsoni*) can be facultative parasites and are pathogens of humans and animals (Alimov, 2000).

There are species dwelling in fresh and sea water and in soil (Alimov, 2000).

The amoebae we found are doubtlessly acanthopodial (fig. 6). In locomotion, the protist becomes a triangle with a wide anterior end. The frontal area of the hyaloplasm produces thin subpseudopodia. The nucleus is near the anterior end of the cell. The length of the locomotive form is 12 to 18 μm, the width is 4.5–6.5 μm, and the L/B ratio is 1.5. The diameter of the nucleus is 1.5–1.7 μm. We observed cyst formation in culture (fig. 6, *9*). The ectocyst is smooth, joined to the endocyst at the tips of the arms. The endocyst is stellate with 5–6 arms. Cyst diameter is up to 18 μm. The species-level identification of *Acanthamoeba* is carried out using data on culture conditions, virulency and enzyme analysis (Pussard, Pons, 1977; Dagget et al., 1982). The morphological observations allowed the organism to be identified as *Acanthamoeba* sp.

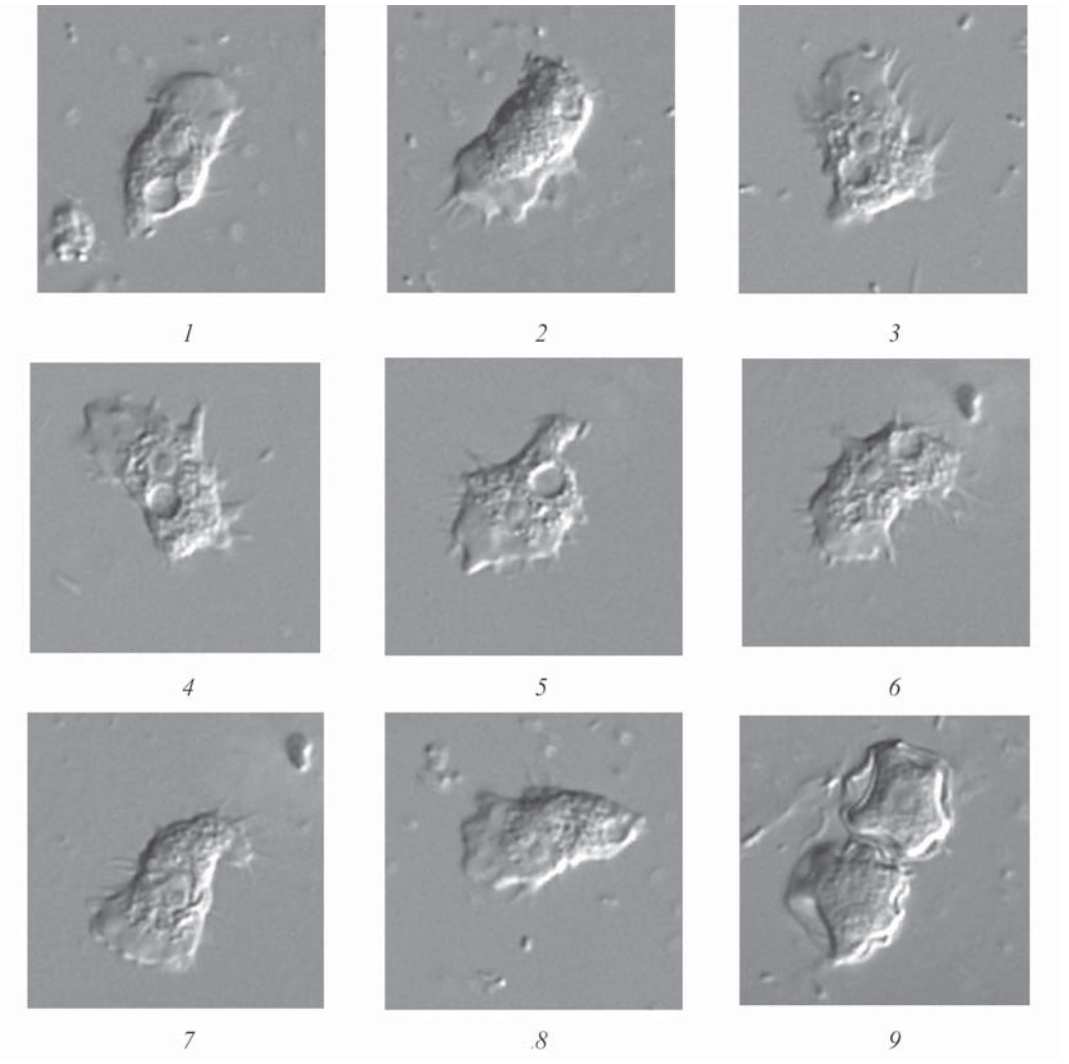

Fig. 6. *Acanthamoeba* sp.: *1–8* — locomotor forms; *9* — cysts, ×1240.

The species is first reported from the river Guyva (Andrushivka District) and the river Uzh (Korosten District) in Zhytomyr Region.

Class HETEROLOBOSEA Page et Blanton, 1985
Family VAHLKAMPFIIDAE Jollos, 1917
Genus *Vahlkampfia* Chatton et Lalung-Bonnaire, 1912

The species belonging to the genus *Vahlkampfia* are of the eruptive morphotype. It is a group of small limax amoebae which move fast and constantly change the direction of their locomotion. Uroidal structures are absent. Most species have single nuclei and are capable of forming cysts.

They live in fresh and salt water and in soil (Alimov, 2000).

Vahlkampfia spp. are known from the water bodies of Europe, Antarctica, North America (Page, Siemensma, 1991).

The amoebae found were rather small and belonged to the eruptive morphotype (fig. 7). The cytoplasm moves fast, eruptively. The wave of the hyaloplasm rolls along all sides of the cell. The process is followed by the loss of the monopodial shape. When in motion, the posterior end is drawn to the anterior one, and as a result the cell becomes globular. Definitive

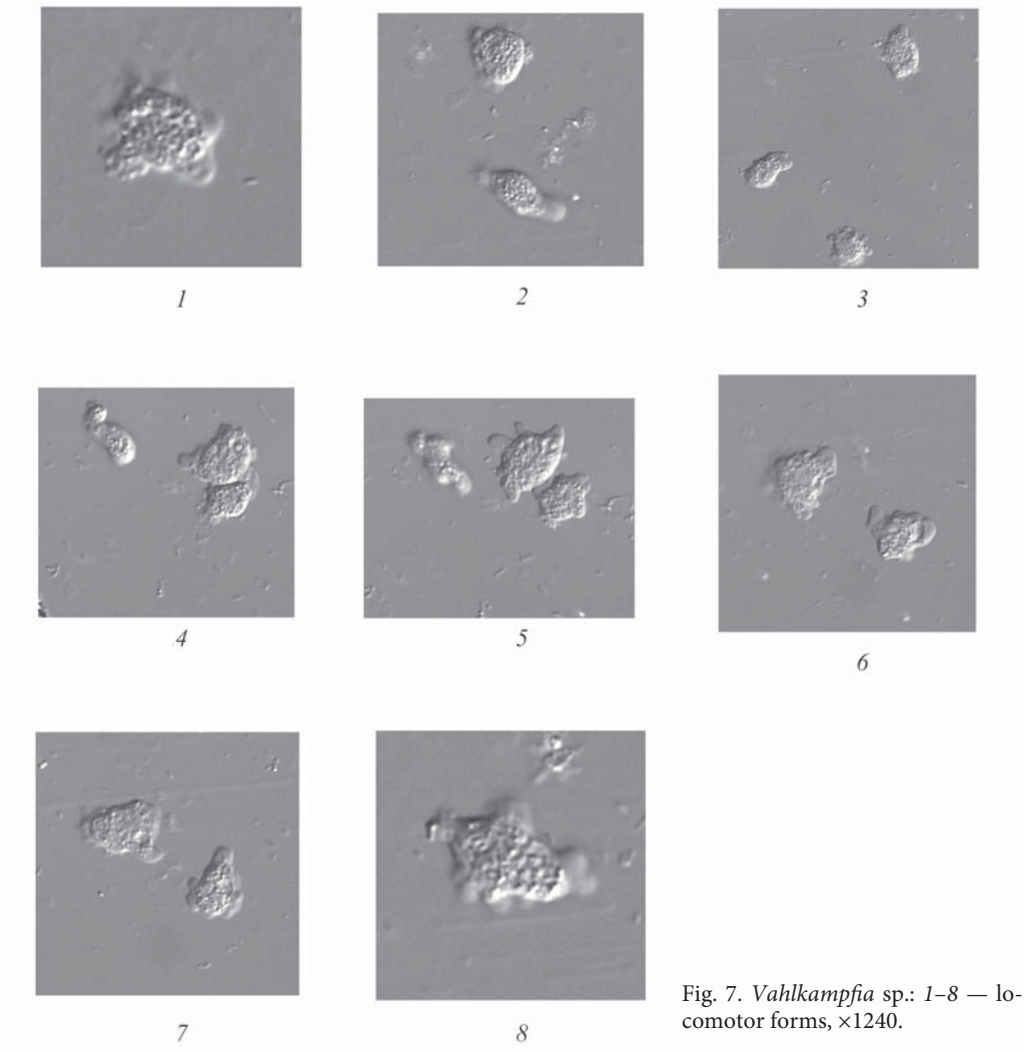

Fig. 7. *Vahlkampfia* sp.: *1–8* — locomotor forms, ×1240.

uroidal structures are absent. The cell is 20–25 μm long, 8–12 μm wide, and the L/B ratio is 1.6–3.0. The single nucleus is 2.4 μm in diameter.

We have previously found the species in the water bodies of Zhytomyr, Kyiv and Volyn Polissya (Patcyuk, Dovgal, 2012; Patsyuk, 2014 c). Here we report a new locality, a river in Korivka village (Kalynivka District, Vinnytsia Region).

The species of amoebae found in the present study in the water bodies of Ukraine (*Saccamoeba* sp., *Ripella* sp., *V. lata*, *Thecamoeba* sp., *Acanthamoeba* sp., and *Vahlkampfia* sp.) belong to monotactic, fan-shaped, striate, acanthopodial and eruptive morphotypes. For most of these amoebae, morphological data are not sufficient to definitely identify the species, so the majority of them were identified to the genus level herein. The list of naked amoebae of Ukraine was complemented by two new species: *Acanthamoeba* sp. and *Thecamoeba* sp., which had not been registered in previous studies, and one new morphotype — the acanthopodial one.

Additional studies involving molecular methods are necessary for exact species identification of the amoebae found. The studies might include the investigation of 18S rRNA gene that was reported to be a variable locus in amoebae (Smirnov, 2011). Besides, the Cox1 mitochondrial gene was proposed as a universal species marker for amoebae (Kudryavtsev, 2014).

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