Vannella melangica sp. nov. (Amoebozoa, Discosea, Vannellidae) from the biogumus produced by *Zophobas morio* larvae

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Summary

One of the modern methods of processing organic wastes is the use of larvae of insects, feeding on organic mattes of the waste mass and transforming biosubstances in biogumus - an organic matter, widely applied as organic fertilizer. It is suggested that the gut microbiome of larvae plays a key role in this process. There are data on fungal and bacterial compounds of larvae gut microbiome and the resulting biogiumus, but the protozoan community of the biogumus still has low attention. During the study of the amoebae fauna of the biogumus produced by *Zophobas morio* larvae, we isolated a vannellid amoebae, resembling *Vannella pentlandii* and *V. placida*, but showing certain morphological differences and differences in the 18S rRNA gene sequences. We described it as *Vannella melangica* sp. nov. (Amoebozoa, Discosea, Vannellidae).

KEY WORDS: *Vannella*, free-living amoebae, *Zophobas morio*, biogumus, municipal waste, bioconversion, Amoebozoa.

Introduction

Organic municipal waste is widely used as a nutrient substrate for the cultivation of invertebrates, particularly insect larvae and worms (Hoornweg et al., 2013; Lim et al., 2016; Parry et al., 2016). One of the most common methods of processing organic waste matter is the use of earthworms – vermicomposting (Thakur et al. 2021; Vidal et al. 2023) or larvae of insects, most commonly – *Musca domestica*, *Hermetia illucens*, *Tenebrio molitor*, and *Zophobas morio* (Andrade et al., 2021; Sukhodolskaya et al., 2025; Gafarova et al., 2026). The bioconversion of wastes provides as a result an organic matter, known as vermicompost or biogumus, widely applied as organic fertilizer (Theunissen et al., 2010; Thakur et al., 2021). The biomass of worms and larvae can then be used to feed livestock, poultry, fish, and fur-bearing animals (Sabine et al., 1983; Byambas et al., 2019; Musyoka et al., 2019).

Among different methods of bioconversion of the organic waste, the use of *Zophobas morio* larvae is remarkable because these organisms can feed on plastics, in particular – polystyrene and are capable of absorbing long-chain plastic molecules and depolymerizing them into digestible low-molecular-weight compounds (Yang et al., 2020; Rosidi et al., 2025). It is suggested that the gut microbiome plays a key role in this process, as evidenced by a significant reduction in the larval capacity to digest plastic when the microbiota is suppressed by antibiotics (Sun et al., 2022; Urbanek et al., 2024). There are data on fungal and bacterial compounds of larvae gut microbiome, but the protozoan part of this process still has low attention. The diversity, abundance and role of protists, including naked amoebae in the microbiome of insect gut and resulting product – biogumus – remains almost not known.

During investigations of the protozoan microbiome of the biogumus produced by *Z. morio* from the organic municipal wastes, a characteristic fan-shaped amoebae were detected. Molecular and morphological study resulted in the identification of these amoebae as representatives of the genus Vannella. This isolate resembled *Vannella pentlandii* and *V. placida*, but showing certain morphological differences and differences in the 18S rRNA gene sequences. We described it as *Vannella melangica* sp. nov. (Amoebozoa, Discosea, Vannellidae) and discuss some problems of the identification of closely related *Vannella* species.

Material and Methods

Sampling and cultivation. The strain studied was isolated from the sample of biogumus produced by *Zophobas morio* larvae fed on municipal solid wastes, supplied from the landfill of the company Spetstrans No. 1 (St.Petersburg, Russia). To isolate amoebae, small samples (1–2 mm^3) of biogumus material were placed in sterile 60 mm Petri dishes containing PJ medium (Prescott and James, 1955). The cultures were incubated at 12–15° C under ambient laboratory light. Amoebae were detected after two weeks of incubation and were transferred to 60 mm Petri dishes with wMY agar (Spiegel et al., 1995). Clonal cultures were established by excising small agar fragments containing a single, well-isolated amoeba cell. These fragments were then transferred to dishes with fresh agar medium. Cultures were maintained on accompanying bacteria and fungi.

Light microscopy. Live cells were examined and photographed on poly-l-lysine coated glass slides (Thermo Fisher Scientific, USA) using a Leica DM2500 microscope equipped with differential interference contrast (DIC) and a Nikon DS-Fi3 camera controlled by NIS-Elements AR software (Nikon Inc., Japan). Morphometric measurements were conducted on 100 cells on the poly-l-lysine coated glass surface.

DNA extraction, amplification and sequencing. To extract DNA, cells were washed off the agar surface with sterile PJ medium and concentrated in 1.7 ml Eppendorf tubes by centrifugation for 90 s at 1,500 g. Genomic DNA was extracted from the cell pellet using guanidine thiocyanate buffer (Maniatis et al., 1982, p. 189). For PCR amplification of the 18S rRNA gene, we used Thermo Scientific Taq DNA Polymerase (Thermo Fisher Scientific, USA) and universal eukaryotic primers: RibA (5'–ACCTGGTTGATCCTGCCAGT–3'), which represents the second half of the original "Primer A" (Medlin et al., 1988), and the reverse primer RibB (5'–TGATCCTTCTGCAGGTTCACCTAC–3') (Pawlowski, 2000). The thermal cycling parameters were: initial denaturation (10 min at 95°C), followed by 35 cycles of 30 s at 94°C, 1 min at 58°C, and 2 min at 72°C, with a final extension of 10 min at 72°C. The resulting amplicons were purified from a 1.5% agarose gel using the Cleanup Mini purification kit (Evrogen, Moscow, Russia) and sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit with RibA, s6F, s12.2, s12.2R, s14, s20R, and RibB primers (Medlin et al., 1988; Pawlowski, 2000; Adl et al., 2014).

Molecular identification. The resulting sequence was mounted into a Discosea alignment, which included available *Vannella* sequences retrieved from the National Center for Biotechnology Information databases (Benson et al., 2013) and a representative set of

outgroups. The sequences were automatically aligned using the E-INS-i algorithm (Katoh et al. 2005) of MAFFT v.7 (Katoh et al. 2002) using the MAFFT online service (Katoh et al. 2018). The system by Brimacombe (1991) was used as a reference to identify regions and helices in the 18S rRNA gene sequences. The boundaries of variable regions were established according to Mesentsev et al. (2026). Pairwise comparisons of sequences were performed using the PAIRS tool (Mesentsev, 2026). Picture showing the identity level of sequences were prepared and formatted using Microsoft Excel.

The obtained sequence was deposited with GenBank under the number ####### (*Vannella pentlandii* GumVan1, length 2612 bp).

Result and discussion

Identification of strain GumVan1

During active locomotion on a glass surface, amoebae adopted a fan-shaped morphotype (Fig. 1A-D; Smirnov and Goodkov, 1999; Smirnov and Brown, 2004), characterized by a broad anterior hyaline area occupied most of the cell volume, while granuloplasm was bounded to a smaller posterior region. During directed movement, neither discrete pseudopodia nor subpseudopodia were seen. The posterior end was rounded (Fig. 1A and B) or truncate (Fig. 1C and D) and lacked distinct uroidal structures. Thus, when moving, the cells have a rounded or spatulate outline; less often, the cells acquire a longitudinally elongated tongue-shaped form (Fig. 1E and F) or, conversely, a wider fan-shaped form in which the hyaloplasmic zone is, as it were, pressed into the hyaline zone from behind (Fig. 1G). Stationary or slowly moving cells had wavy edges, especially in the hyaline zone (Fig. 1H). In the frontal hyaline zone on the dorsal surface, longitudinal ridges were sometimes observed.

Observations using a light microscope allow us to classify the studied strain as belonging to the family Vannellidae (Discosea; Amoebozoa), since the amoebae exhibit its key diagnostic features: characteristic flattened, fan-shaped locomotive forms, absence of pronounced pseudopodia or subpseudopodia (Page 1988; Smirnov et al., 2007; 2011). Currently, this group includes approximately 50 named species; numerous unnamed isolates are also known. For reliable identification, the complete 2612-nucleotide sequence of the 18S rRNA gene of our isolate was obtained. A BLASTn search using the sequence of the present strain as a query returned the highest similarity with amoebae of the genus *Vannella*, especially with species

forming a “PEF clade” (Maciver et al., 2017). Phylogenetic analysis confirmed our morphological identification and the BLASTn search results: the strain occupied a sister position to a clade including *V. pentlandii*, *V. placida*, *V. fimicola*, and several sequences of uncultured soil organisms (Fig. 2). *V. epipetala* formed a basal branch relative to the aforementioned highly supported clade. Pairwise comparison of sequences revealed that the studied strain is most closely related to *V. pentlandii* (Table 1), which was originally isolated from the soil from the roots of *Capsella bursa pastoris* plants in the Pentland Hills, near Silverburn, Scotland (Maciver et al., 2017).

The sequence discrepancy comprises 13 indels in our strain, which correspond to additional nucleotides in the *V. pentlandii* sequence (GenBank accession number KY344796). Most of the other differences were transitions. There are only two substitutions that are transversions; both are located in the 21st helix, which is part of the V4 variable region.

Morphological features observed in amoebae of the studied strain also align with the diagnosis of *V. pentlandii* and traits of vannellid amoebae from the PEF clade (Sutherland et al., 2017). Characteristic longitude ridges on the surface of moving cells were occasionally seen (Fig. 1B and C). The nucleus had a single near spherical or slightly uneven nucleolus located in the center (Fig. 1M and N). The nucleolus was homogenous and had a smooth surface. Although, some variations in nucleoli forms were rarely seen (Fig. 1O and P). Furthermore, our observations confirmed that these amoebae exhibit poor adhesion to glass surfaces. During our observations of amoebae on temporary preparations, the amoebae almost immediately stopped moving when they entered the field of view (and accordingly, the illuminated field), after which the cells contracted and broke away from the substrate (Fig. 1I and J).

Morphometric analysis showed that sizes of the cells of VanGum1 strain are comparable to those reported in the description of *V. pentlandii*, although there are some differences. In locomotion, amoebae of the strain VanGum1 were on average slightly shorter, with a mean length of 26 µm compared to 28 µm in the diagnosis. The smallest measured cell reached only 18 µm in contrast to a minimum length of 22 µm reported in the literature. Besides that, cells of the strain VanGum1 were wider during locomotion with a mean breadth of 25 µm against 20 µm in the description of *V. pentlandii*. The recorded maximum was also noticeably higher, reaching 36 µm, whereas the maximum recorded width for *V. pentlandii* was 27 µm. Furthermore, while *V. pentlandii* was characterized by a relatively small nucleus measuring only

3-4 µm in diameter, the diameter of nuclei of meausered cells from the studied strain ranged from 3 to 8 µm, with an average of 5 µm.

The cells of VanGum1 strain display several differences from the original description of *V. pentlandii*. While *V. pentlandii* was characterised by the presence of «pronounced tail» during locomotion, we observed no such structure. We suppose that this «tail» described in the literature may be a cytoplasmic projection from the floating form that has not yet been fully retracted after attachment to the substrate. Besides that, while the original description of V. pentlandii includes only one type of floating form, amoebae of VanGum1 strain exhibited two types, which differed primarily in the length and thickness of their hyaline projections (Fig. 1I-L). In the first case, the amoebae had smooth, thick, finger-like processes, the base of which was usually filled with granuloplasm. In the second case, the amoebae had a compact, almost spherical body, from which extended about ten thin projecctions filled only with hyaloplasm. The length of these projections was approximately equal to the diameter of the central body mass. Cysts were not present in our cultures, in contrast with the original description. The lack of cysts could also be explained by specific cultivation factors that simply did not trigger the process of encystment. Based on the results of the morphological comparison and sequence differences, the present isolate should be described as a new species.

Taxonomic summary

Vannella melangica sp. nov. Mesentsev, Kolomyttseva et Smirnov, 2026

Diagnosis: During locomotion, amoebae are flattened, with subcircular or, less frequently, elongated outlines. Length in locomotion is 17–36 µm; length/breadth ratio is 0.5–2.0. Cell periphery smooth or slightly wavy. Posterior end rounded, convex, or flat, entirely lacking uroidal structures. One or two faint longitudinal ridges occasionally appear on the dorsal surface within the hyaloplasmic zone. Floating forms exhibit dimorphism, occurring as two distinct morphotypes: (1) an irregularly shaped central mass with finger-like pseudopodia that may branch at the base and often contain granuloplasm proximally; and (2) compact, spherical cells bearing about a dozen thin, curved, purely hyaloplasmic pseudopodia. Nucleus is single with a centrally located, spherical nucleolus; diameter of nucleus is 3-8 µm; dimeter of nucleolus is 1-6 µm.

Differences from closely related species: *V. melangica* sp. nov. differs from *V. placida* in the more frequent formation of longitudinal ridges, and from *V. pentlandii* by the absence of a "tail" (an elongated posterior end). *V. melangica* sp. nov. can also be identified by its 18S rRNA gene sequence, specifically at the beginning of the V4 variable region, which contains the unique motif gaagtttaattggattggtccac (649–671 bp).

Type material: The holotype on the Heidenhain's iron hematoxylin-stained permanent preparation of the strain GumVan1 was deposited with the Collection of slides of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology RAS, under the No ####.

Ethymology: The specific epithet melangica (L. fem. adj.) refers to "melange" (also known as the spice), the fictional, most valuable substance in Frank Herbert's sci-fi novel Dune. This name highlights the ecological context of the isolation source: the amoebae were obtained from granules of biogumus produced by *Zophobas morio* larvae. These worm-like creatures ("superworms") are gigantic compared to the amoebae, mirroring the ecosystem of the planet Arrakis, where giant desert worms and their larvae are uniquely responsible for the generation of melange.

Notes on the observations of *Vannella* in various sorts of environment

The presence of *Vannella melangica* sp. nov. amoebae in biogumus produsedby *Zophobas* larvae from MSW is consistent with the biological characteristics of other members of this phylogenetic lineage (Fig. 2). The clade to which the studied amoeba belongs primarily comprises species found in terrestrial habitats (Palataicova et al., 2026). For example, *V. epipetala* was first isolated from *Spondias mombin* "leaf wash" (Amaral-Zettler et al., 2006), *V. pentlandii* was described from soil samples (Maciver et al., 2017), and *V. placida* was discovered in storm sewer (Page, 1968). Additionally, metatranscriptomic analyses of the oat rhizosphere aimed at understanding RNA virus distribution identified three sequences clustered within this clade (Starr et al., 2019). The group also includes *V. fimicola*, a species originally isolated from cow and goat dung samples (Olive, 1962). However, the lack of detailed sampling metadata makes it difficult to determine whether *V. fimicola* colonized the dung directly from the environment or passed intact through the animal's digestive tract.

In addition to their specific isolation sites, the representatives of this clade exhibit distinct ecological and structural adaptations. Their diverse habitats share a common feature:

they undergo variable moisture regimes with alternating dry and wet periods, differing significantly from freshwater environments. The primary mechanism enabling survival under such fluctuating terrestrial conditions is the capacity to encyst, a trait shared by all representatives of this clade, although not unique among vannellids (Maciver et al., 2017). A remarkable example of this adaptation is found in *V. fimicola*, which displays a specialized encystment mechanism that forms single-celled "fruiting bodies" (Olive, 1962; Shadwick et al., 2009). These cysts are elevated above the substrate on a stalk – a structural modification hypothesized to facilitate efficient environmental dispersal via wind or small invertebrates. Therefore, given that this group represents a specialized terrestrial lineage adapted to fluctuating moisture levels, we hypothesize that *V. melangica* sp. nov. can maintain metabolic activity and phagocytose bacteria in vermicompost even under short-term, low-humidity conditions. This interaction may potentially ensure bacterial survival within either the trophozoite or the cyst during unfavorable periods, consistent with observations in other free-living amoebae (Scheid, 2014; Rubenioa et al., 2017).

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Tables

Sequence/ species	**1.**	2.	3.	4.	5.
1. *V. melagica* sp. nov. VanGum1	100	99,03	98,29	98,34	95,4
2. KY344796 *V. pentlandii*	97,49	100	97,44	97,49	94,66
3. AY294150 *V. placida*	96,18	95,44	100	97,38	94,27
4. FJ766470 *V. fimicola*	95,71	94,34	94,81	100	94,6
5. DQ913094 *V. epipetala*	90,45	88,75	88,44	89,19	100

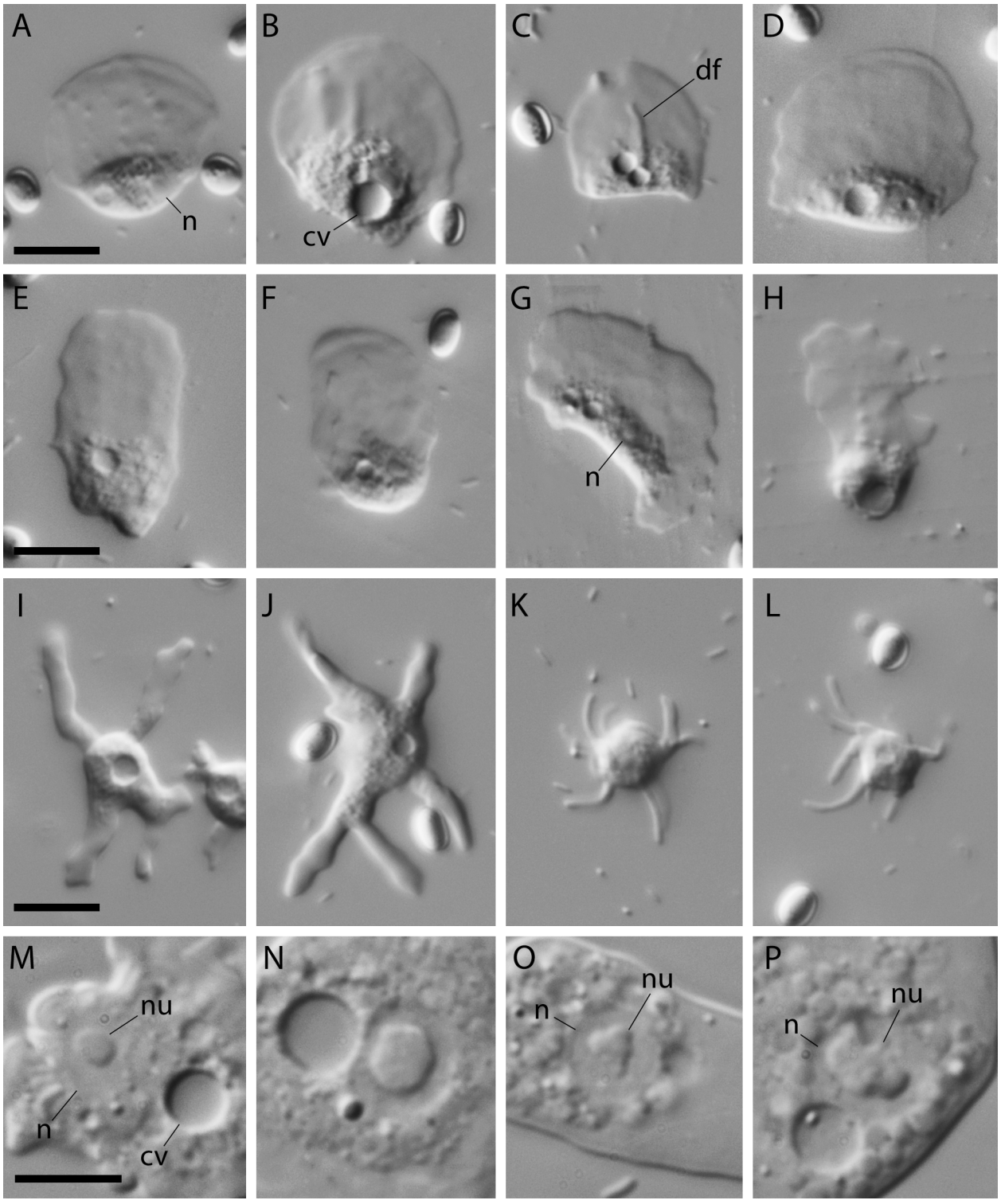
Table 1. Levels of sequence identity (lower-left triangle) and similarity (upper-right triangle) for the 18S rRNA gene fragments within the *Vannella* spp. (PEF clade). Column headers correspond to the sequence numbers listed on the left. 1850 sites.

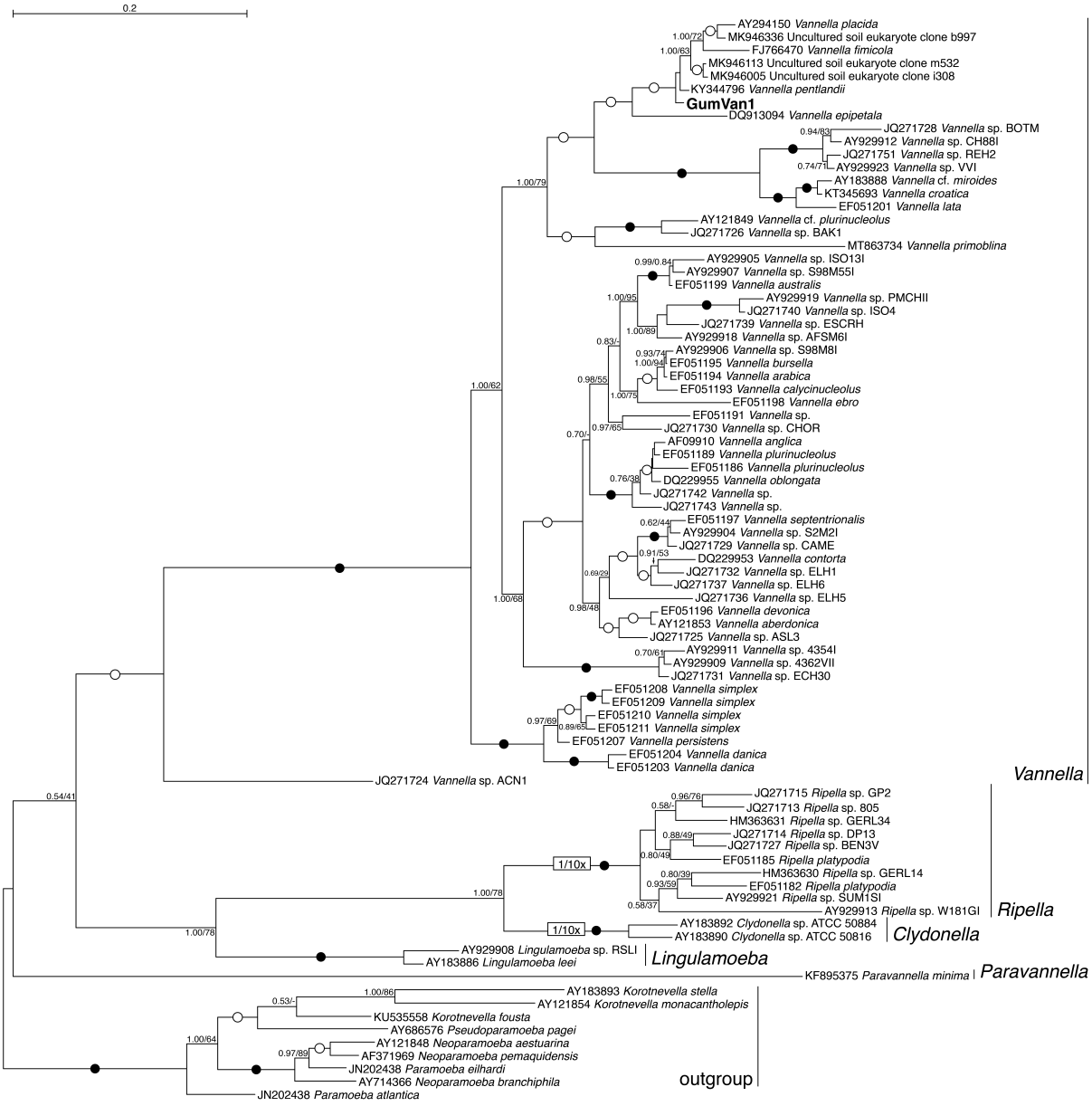
435 **Legends to Figures**

436 **Fig. 1.** Light microscopy of *Vannella melangica* strain, DIC. A and B – rounded locomotive
437 forms. C and D – spatulate locomotive forms. E and F – elongated locomotive forms. I-L –
438 floating forms; M and N – nuclei; O and P – unusual nuclei. *Abbriviations*: cv – contractile
439 vacuole; df – dorsal fold; n – nucleus; nu – nucleolus. Scale bars: A–L – 10 µm; M–P – 5 µm.

440 **Fig. 2.** Maximum-likelihood (IQ-TREE) phylogenetic tree of strains assigned to *Vannella*
441 spp., rooted with Dactilopodida species, based on SSU rRNA gene sequences. 1567 sites used in
442 the analysis. Labeling of nodes: bootstrap value/posterior probability. Black dots indicate fully
443 supported nodes (100/1.00 support); circles indicate highly supported nodes (both BS > 95 and
444 PP > 0.95). Supports lower 50/0.5 are not shown.

445


A
n
B
cv
C
df
D
E
F
G
n
H
I
J
K
L
M
nu
n
cv
N
O
nu
n
P
nu
n


0.2
AY294150 Vannella placida
MK946336 Uncultured soil eukaryote clone b997
FJ766470 Vannella fimicola
MK946113 Uncultured soil eukaryote clone m532
MK946005 Uncultured soil eukaryote clone i308
KY344796 Vannella pentlandii
GumVan1
DQ913094 Vannella epipetala
JQ271728 Vannella sp. BOTM
AY929912 Vannella sp. CH88I
JQ271751 Vannella sp. REH2
AY929923 Vannella sp. VVI
AY183888 Vannella cf. miroides
KT345693 Vannella croatica
EF051201 Vannella lata
AY121849 Vannella cf. plurinucleolus
JQ271726 Vannella sp. BAK1
MT863734 Vannella primoblina
AY929905 Vannella sp. ISO13I
AY929907 Vannella sp. S98M55I
EF051199 Vannella australis
AY929919 Vannella sp. PMCHII
JQ271740 Vannella sp. ISO4
JQ271739 Vannella sp. ESCRH
AY929918 Vannella sp. AFSM6I
AY929906 Vannella sp. S98M8I
EF051195 Vannella bursella
EF051194 Vannella arabica
EF051193 Vannella calycinucleolus
EF051198 Vannella ebro
EF051191 Vannella sp.
JQ271730 Vannella sp. CHOR
AF09910 Vannella anglica
EF051189 Vannella plurinucleolus
EF051186 Vannella plurinucleolus
DQ229955 Vannella oblongata
JQ271742 Vannella sp.
JQ271743 Vannella sp.
EF051197 Vannella septentrionalis
AY929904 Vannella sp. S2M2I
JQ271729 Vannella sp. CAME
DQ229953 Vannella contorta
JQ271732 Vannella sp. ELH1
JQ271737 Vannella sp. ELH6
JQ271736 Vannella sp. ELH5
EF051196 Vannella devonica
AY121853 Vannella aberdonica
JQ271725 Vannella sp. ASL3
AY929911 Vannella sp. 4354I
AY929909 Vannella sp. 4362VII
JQ271731 Vannella sp. ECH30
EF051208 Vannella simplex
EF051209 Vannella simplex
EF051210 Vannella simplex
EF051211 Vannella simplex
EF051207 Vannella persistens
EF051204 Vannella danica
EF051203 Vannella danica
JQ271724 Vannella sp. ACN1
Vannella
JQ271715 Ripella sp. GP2
JQ271713 Ripella sp. 805
HM363631 Ripella sp. GERL34
JQ271714 Ripella sp. DP13
JQ271727 Ripella sp. BEN3V
EF051185 Ripella platypodia
HM363630 Ripella sp. GERL14
EF051182 Ripella platypodia
AY929921 Ripella sp. SUM1SI
AY929913 Ripella sp. W181GI
Ripella
AY183892 Clydonella sp. ATCC 50884
AY183890 Clydonella sp. ATCC 50816
Clydonella
AY929908 Lingulamoeba sp. RSLI
AY183886 Lingulamoeba leei
Lingulamoeba
KF895375 Paravannella minima
Paravannella
AY183893 Korotnevella stella
AY121854 Korotnevella monacantholepis
KU535558 Korotnevella fousta
AY686576 Pseudoparamoeba pagei
AY121848 Neoparamoeba aestuarina
AF371969 Neoparamoeba pemaquidensis
JN202438 Paramoeba eilhardi
AY714366 Neoparamoeba branchiphila
JN202438 Paramoeba atlantica
outgroup