

**Supplementary information**

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**Microbial predators form a new supergroup of eukaryotes**

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## 1 **Supplementary Discussion**

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3 Morphological and phylogenetic data for the isolated clones allow us to recognize three new  
4 genera: a new genus *Ubysseya* with one new species, a new genus *Nibbleromonas* with four new  
5 species, and a new genus *Nebulomonas* with one species. The *Ubysseya* and *Nibbleromonas*  
6 species form a well-supported group, which we name Nibbleridia, while *Nebulomonas* groups with  
7 the previously described *Ancoracysta twisti*<sup>3</sup>, forming another novel group, which we name  
8 Nebulidia. Nibbleridia and Nebulidia form a strongly supported monophyletic group, termed here  
9 Provora (devouring voracious protists).

10 The independent branching position of Provora and unique morphology of provorans  
11 strongly suggest recognition of a novel eukaryotic supergroup. The phylogenetic position of  
12 Provora found here via multigene analysis makes it equivalent in rank to the major supergroups of  
13 eukaryotes. So, here we formally create a name Provora for new supra-kingdom-level group of  
14 eukaryotes to accommodate the newly described diversity of flagellated protists.

15 Supergroup Provora includes two groups Nibbleridia and Nebulidia, classified here as  
16 novel phyla. We also established the new family Nibbleridae, order Nibbleridida, and class  
17 Nibbleridea as well as the new family Nebulidae, order Nebulidida, and class Nebulidea to avoid  
18 future taxonomical confusion based on the likelihood that other representatives of Nibbleridia  
19 and Nebulidia with different morphology and molecular phylogenetic placement will be  
20 discovered.

21 Taxonomic diagnoses of the newly described taxa are given below.

22

### 23 **Taxonomic summary**

24 **Provora** nom. nov. Tikhonenkov et al. 2022

25 Assignment. Eukaryota

26

27 The least inclusive clade containing *Nibbleromonas quarantinus* Tikhonenkov, Belyaev,  
28 Karpov, Zagumyonnyi, Borodina, Prokina, et Keeling 2022, *Ubysseya fretuma* Tikhonenkov,  
29 Belyaev, Karpov, Zagumyonnyi, Borodina, Prokina, et Keeling 2022 (both Nibbleridia) and  
30 *Nebulomonas marisrubri* (Mylnikov and Tikhonenkov 2009) Tikhonenkov, Belyaev, Karpov,  
31 Zagumyonnyi, Borodina, Prokina, Aleoshin, et Keeling 2022, *Ancoracysta twisti* Janouškovec,  
32 Tikhonenkov, Burki, Howe, Rohwer, Mylnikov et Keeling 2017 (both Nebulidia). This is a  
33 node-based definition in which all of the specifiers are extant.

34 The apparent composition of Provora is Nibbleridia (*Nibbleromonas*, *Ubysseya*) and  
35 Nebulidia (*Nebulomonas*, *Ancoracysta*) which are represented by free-living eukaryovorous  
36 heterotrophic bi-flagellates with ventral groove and extrusomes.

37 Etymology. From Eng. protists, devouring, and voracious, indicating the predatory life  
38 style of the representatives.

39 **Phylum Nibbleridia** nom. nov. Tikhonenkov et al. 2022

40 Assignment. Eukaryota; Provora.

41 Free-living unicellular protists with two subapical heterodynamic acronematic flagella  
42 facing to the ventral side and cytostomal ventral groove. Flagella originate from the separate  
43 flagellar pockets above the ventral groove. Posterior flagellum bears two opposite longitudinal  
44 folds. Cell coverings are complex and multilayered with alveolar vesicles. Extrusive organelles  
45 of trichocyst type. Mitochondria with tubular to sac-like cristae. Predators, capture eukaryotic  
46 prey.

47 **Class Nibbleridea** cl. nov. Tikhonenkov et al. 2022

48 **Order Nibbleridida** ord. nov. Tikhonenkov et al. 2022

49 **Family Nibbleridae** fam. nov. Tikhonenkov et al. 2022

50 urn:lsid:zoobank.org:act: 9EE01A01-E294-415B-A36F-0FB4373183D0

51 Diagnoses as for phylum Nibbleridia.

52 Type genus *Nibbleromonas*.

53 *Nibbleromonas* gen. nov. Tikhonenkov et al. 2022

54 Assignment. Eukaryota; Provora; Nibbleridia; Nibbleridea; Nibbleridida; Nibbleridae

55 Diagnosis. Fast swimming sickle-shaped flagellates with distinct thorn under the ventral  
56 groove. Ventral groove is reinforced with bands of microtubules and involved in biting off parts  
57 of the cells of the eukaryotic prey. Cell coverings on the dorsal side of the cell contain 1–2 layers  
58 of alveolar vesicles beneath the cell membrane. Plasma membrane invaginations or micropores  
59 situated between the alveoli. Flagellar transition zone contains a transition cylinder distal to the  
60 transverse plate. Posterior flagellum bears two opposite longitudinal folds. Mitochondrial cristae  
61 with filamentous inclusion.

62 Etymology. From Eng. ‘nibbler’ and monas (lat.) – unicellular organism.

63 Zoobank Registration. urn:lsid:zoobank.org:act:A54BD0FB-7FA3-42CB-9D3D-  
64 2211FA657DC0

65 Type species. *Nibbleromonas quarantinus*.

66

67 *Nibbleromonas quarantinus* sp. nov. Tikhonenkov, Belyaev, Karpov, Zagumyonnyi,  
68 Borodina, Prokina, et Keeling 2022

69 Diagnosis. Cells 3.3–6.2  $\mu\text{m}$  long, 1.6–5.0  $\mu\text{m}$  wide. Anterior flagellum (6.0–9.8  $\mu\text{m}$ ) is  
70 slightly shorter than posterior (10.2–14.5  $\mu\text{m}$ ). Both flagella without mastigonemes. Large  
71 digestive vacuole occupies a significant part of the entire cell volume after feeding.

72 Reproduction by simple binary division, the cells cleaving laterally in an anterior-posterior  
73 direction. No cysts.

74 Type material. A block of chemically fixed resin-embedded cells of the type strain, Colp-  
75 41, is deposited in Marine Invertebrate Collection, Beaty Biodiversity Museum, University of  
76 British Columbia as MI-PR154. This constitutes the name bearing type of the new species (a  
77 hapantotype).

78 Type strain. Colp-41. Storing in the collection of live protozoan cultures at IBIW RAS.

79 Type Figure. Fig. 1g illustrates a live cell of strain Colp-41.

80 Type locality. Silty sand in shoreland of Quarantine Bay, Sevastopol city, Crimea.

81 Etymology. Named after the Quarantine Bay where species was found.

82 Gene sequence. The 18S rRNA gene sequence has the GenBank Accession Number

83 OP102009.

84 Zoobank Registration. urn:lsid:zoobank.org:act:F6395E20-7BDF-4CBE-95FB-

85 E4CE1E7B8185

86

87 *Nibbleromonas curacaus* sp. nov. Tikhonenkov et Keeling 2022

88 Diagnosis. Cells are 3.4–7.1  $\mu\text{m}$  in length, 2.5–4.7  $\mu\text{m}$  in width with anterior nucleus. Posterior

89 flagellum longer than anterior, and both are longer than the cell. Sated cells are wide-oval with

90 prominent posterior food vacuole. Flagella and their beating are almost invisible when the cell is

91 moving. Cells rotate in different planes at swimming with frequent changes of the direction of

92 movement. Cysts not found.

93 Type Figure. Fig. 1f illustrates a live cell of strain Cur-12.

94 Type locality. Scraping from the sponges at the eastern point of the Curaçao island.

95 Etymology. Named after the Curaçao island where species was found.

96 Gene sequence. The 18S rRNA gene sequence has the GenBank Accession Number

97 OP102003.

98 Zoobank Registration. urn:lsid:zoobank.org:act:F1E8545D-BAC1-44FF-9B6B-

99 8FEE4AC028BB

100

101 *Nibbleromonas kosolapovi* sp. nov. Tikhonenkov, Belyaev, Karpov, Zagumyonnyi,

102 Borodina, Prokina, et Keeling 2022

103 Diagnosis. Cells are 3.3–6.7  $\mu\text{m}$  in length, 2.2–5.6  $\mu\text{m}$  in width. Sated cells are

104 matryoshka-shaped, with a distinctly shaped digestive vacuole located in the cell posteriorly.

105 Anterior flagellum (4.4–5.4  $\mu\text{m}$ ) is slightly shorter than posterior (6.45–9.45  $\mu\text{m}$ ), both without  
106 mastigonemes. Flagella can wrap around the cell. When moving forward, the cell makes a  
107 spindle-shaped movement around its axis. Cysts not found.

108 Type strain. Colp-32. Storing in the collection of live protozoan cultures at IBIW RAS.

109 Type Figure. Fig. 1e illustrates a live cell of strain Colp-32.

110 Type locality. Arctic waters of the Kara Sea.

111 Etymology. Named after Dr. Natalia Kosolapova, Russian protistologist, who carried out  
112 fieldwork and collect samples, where new species was found.

113 Gene sequence. The 18S rRNA gene sequence has the GenBank Accession Number  
114 OP102007.

115 Zoobank Registration. [urn:lsid:zoobank.org:act:66A5C066-890F-4F25-AAB6-](https://zoobank.org/act:66A5C066-890F-4F25-AAB6-5CDCE2028034)  
116 [5CDCE2028034](https://zoobank.org/act:66A5C066-890F-4F25-AAB6-5CDCE2028034)

117

118 *Nibbleromonas arcticus* sp. nov. Tikhonenkov, Belyaev, Karpov, Zagumyonnyi, Borodina,  
119 Prokina, et Keeling 2022

120 Diagnosis. Cells are 2.7–5.3  $\mu\text{m}$  in length, 1.7–5.3  $\mu\text{m}$  in width. Anterior flagellum (4.5–  
121 6.9  $\mu\text{m}$ ) is slightly shorter than posterior (6.2–9.0  $\mu\text{m}$ ), both without mastigonemes. Cells divide  
122 laterally in an anterior-posterior direction. Cysts not found.

123 Type strain. Colp-45. Storing in the collection of live protozoan cultures at IBIW RAS.

124 Type Figure. Fig. 1d illustrates a live cell of strain Colp-45.

125 Type locality. Arctic waters of the East Siberian Sea.

126 Etymology. Species epithet reflects the place of finding in the Arctic Ocean.

127 Gene sequence. The 18S rRNA gene sequence has the GenBank Accession Number  
128 OP101999.

129 Zoobank Registration. [urn:lsid:zoobank.org:act:830A4372-62D9-4CE1-BFD8-](https://zoobank.org/act:830A4372-62D9-4CE1-BFD8-9FE9EED67FED)  
130 [9FE9EED67FED](https://zoobank.org/act:830A4372-62D9-4CE1-BFD8-9FE9EED67FED)

131

132 *Ubysseya* gen. nov. Tikhonenkov et al. 2022

133 Assignment. Eukaryota; Provora; Nibbleridia; Nibbleridea; Nibbleridida; Nibbleridae

134 Diagnosis. Fast swimming flagellates with inconspicuous ventral groove. Cells are

135 roundish, rarely oval. Space between two flagellar pockets is reinforced with a band of

136 microtubules. Ventral groove is reinforced with bands of microtubules. Cell coverings have

137 unstable structure varying from single plasma membrane to alveoli with vesicles and

138 multimembranes. Cytopharynx is present. Flagellar transition zone contains a transition cylinder

139 distal to the curved transverse plate.

140 Etymology. Derived from Eng. UBC (University of British Columbia) and 'The Ubyssesey' -

141 UBC's official student newspaper.

142 Zoobank Registration. urn:lsid:zoobank.org:act:DFE7080B-6201-455A-99CE-

143 903103CBB049

144 Type species. *Ubysseya fretuma*.

145

146 *Ubysseya fretuma* sp. nov. Tikhonenkov, Belyaev, Karpov, Zagumyonnyi, Borodina,

147 Prokina, et Keeling 2022

148 Diagnosis. Cells are 3.4–6.3  $\mu\text{m}$  long, 2.8–4.8  $\mu\text{m}$  wide. Anterior flagellum (6.9–9.1  $\mu\text{m}$ ) is

149 slightly shorter than posterior (9.1–11.6  $\mu\text{m}$ ). Both flagella without mastigonemes. Cells spin

150 around their longitudinal axis then swimming. Flagella of sated cells wrap around the cell body.

151 Longitudinal division. Cysts not found.

152 Type material. A block of chemically fixed resin-embedded cells of the type strain, TD-3,

153 is deposited in Marine Invertebrate Collection, Beaty Biodiversity Museum, University of British

154 Columbia as MI-PR155. This constitutes the name bearing type of the new species (a

155 hapantotype).

156 Type strain. TD-3. Storing in the collection of live protozoan cultures at IBIW RAS.

157 Type Figure. Fig. 1a illustrates a live cell of strain TD-3.

158 Type locality. Water column of the Strait of Georgia, British Columbia, Canada.

159 Etymology. From fretum (lat.), strait, channel.

160 Gene sequence. The 18S rRNA gene sequence has the GenBank Accession Number

161 OP102006.

162 Zoobank Registration. urn:lsid:zoobank.org:act:A230EC14-DC4B-4F05-8D69-

163 8FE0BAB3DE09

164

165 **Phylum Nebulidia** nom. nov. Tikhonenkov et al. 2022

166 Assignment. Eukaryota; Provora.

167 Free-living unicellular protist with two subapical non-arconematic heterodynamic flagella

168 inserted into separate flagellar pockets. Posterior flagellum with a short lateral vane associated

169 with shallow and not long ventral groove supported by microtubules. Cortical alveolar system

170 underlies the entire cell surface except the flagellar pockets and cytopharynx. Extrusive

171 organelles of ancoracyst and toxicyst types. Predators, capture eukaryotic prey.

172

173 **Class** Nebulidea cl. nov. Tikhonenkov et al. 2022

174 **Order** Nebulidida ord. nov. Tikhonenkov et al. 2022

175 **Family** Nebulidae fam. nov. Tikhonenkov et al. 2022

176 urn:lsid:zoobank.org:act:B8894608-40D4-4D16-A4D9-6F448614F22C

177 Diagnoses as for phylum Nebulidia.

178 Type genus *Ancoracysta* Janouškovec, Tikhonenkov, Burki, Howe, Rohwer, Mylnikov et

179 Keeling 2017

180 ***Nebulomonas*** gen. nov. Tikhonenkov et al. 2022

181 Assignment. Eukaryota; Provora; Nebulidia; Nebulidea; Nebulidida; Nebulidae

182 Diagnosis. As for phylum Nebulidia. Extrusive organelles of toxicyst type.



183 Etymology. From Latin nebulosus (misty) and monas (lat.) – unicellular organism.  
184 Zoobank Registration. urn:lsid:zoobank.org:act:97B89F6F-72D6-482A-9EA7-  
185 88E5C63E6EB6  
186 Type species. *Nebulomonas marisrubri*.  
187  
188 *Nebulomonas marisrubri* comb. nov. Tikhonenkov, Belyaev, Karpov, Zagumyonnyi,  
189 Borodina, Prokina, et Keeling 2022  
190 basionym *Colponema marisrubri* Mylnikov and Tikhonenkov 2009  
191 synonym *Ancoracysta marisrubri* Cavalier-Smith 2018