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Analysis of the Dinoflagellate *Prorocentrum minimum* Transcriptome: Identifying the Members of the Voltage-Gated Cation Channel Superfamily[1]

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Abstract—Dinoflagellates are an ecologically important group of aquatic single-cell eukaryotes. At present, relatively little is known about physiological features that determine the role of these protists in natural ecosystems. The lack of knowledge on the diversity, structure, and functioning of dinoflagellate ion channels significantly hampers the interpretation of physiological reactions and adaptations in these microorganisms. We performed the analysis of the translated transcriptome databases that belong to two strains of the dinoflagellate *Prorocentrum minimum* in order to identify the members of the voltage-gated cation channel superfamily. We found out that transcriptomes of these potentially toxic microorganisms contained the homologues of: 1—inwardly rectifying potassium channels (K_{ir}), 2—voltage-gated potassium channels (K_v), 3—calcium-activated potassium channels (K_{Ca}), 4—cyclic nucleotide-gated channels (EAG and HCN/CNG), 5—TRPV and TRPP channels, 6—two-pore calcium channels TPC, 7—voltage-gated sodium (Na_v) and calcium (Ca_v) channels, 8—and voltage-gated proton channels (H_v).

Keywords: unicellular eukaryotes, protists, dinoflagellates, ion channels, transcriptome

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Dinoflagellates are unicellular eukaryotic organisms that play a crucial role in functioning of aquatic ecosystems. These protists are well known for their ability to produce a great variety of secondary metabolites which are often toxic for animals and humans. Many dinoflagellate species are able to form the so-called harmful algal blooms, or "red tides". The blooms of toxic species lead to the accumulation of toxins in mussels, fish and other aquatic animals and thus cause damage to public health and economy of the coastal regions (Cembella, 2003; Okolodkov, 2011). Thus, dinoflagellates play a variety of ecologically important roles.

Dinoflagellates possess a number of unique cytological and biochemical features, such as 1—the permanently condensed chromosomes during the cell cycle; 2—the special type of mitosis (dinomitosis) during which the spindle microtubules within the cytoplasmic channels run through the dividing nucleus; 3—one of the biggest genomes among eukaryotes (up to 250 pg/cell); 4—biosynthesis of unusual sterols (dinosterols); 5—bioluminescence; 6—combination of auto- and heterotrophy (mixotrophy); 7—a complex chloroplasts that originated as a result of tertiary endosymbiosis in the process of evolution, etc. (Raikov, 1995; Soyer-Gobillard et al., 1999; Okolodkov, 2011; Figueroa et al., 2014). At the same time, many aspects of dinoflagellate physiology are still poorly studied. This fact hampers our understanding of ecological and toxicological impacts of this highly relevant group of organisms as well as the interpretation of the accumulated data on their general biology.

The role of ion channels in a wide range of physiological processes in animal and plant cells has been described in details (Hille, 2001; Krutetskaya et al., 2003; Zefirov and Sitdikova, 2010). Ion channels are essential components of the cell signaling and are involved in the processes of adaptation, proliferation, differentiation, cell motility, exo- and endocytosis, etc. Although ion channels of dinoflagellates are poorly investigated, it is reasonable to assume their high relevance to this group of eukaryotes. For instance, voltage-activated sodium, proton, and chlorine currents important for the action potential devel-

[1] The article was translated by the authors.

Abbreviations: a. a.—amino acid residues, Ank—ankyrin domain, BK—large conductance calcium-activated potassium channel, Ca_v—voltage-gated calcium channel, CNBD—cyclic nucleotide-binding domain, CNG—cyclic nucleotide-gated channel, EAG—ether-a-go-go like channel, HCN—hyperpolarization-activated cyclic nucleotide-gated channel, K_{2P}—potassium leak channel, K_{ir}—inwardly rectifying potassium channel, K_v—voltage-gated potassium channel, NALCN—sodium leak channel, Na_v—voltage-gated sodium channel, P-loop—pore loop, VSD—voltage-sensitive domain.

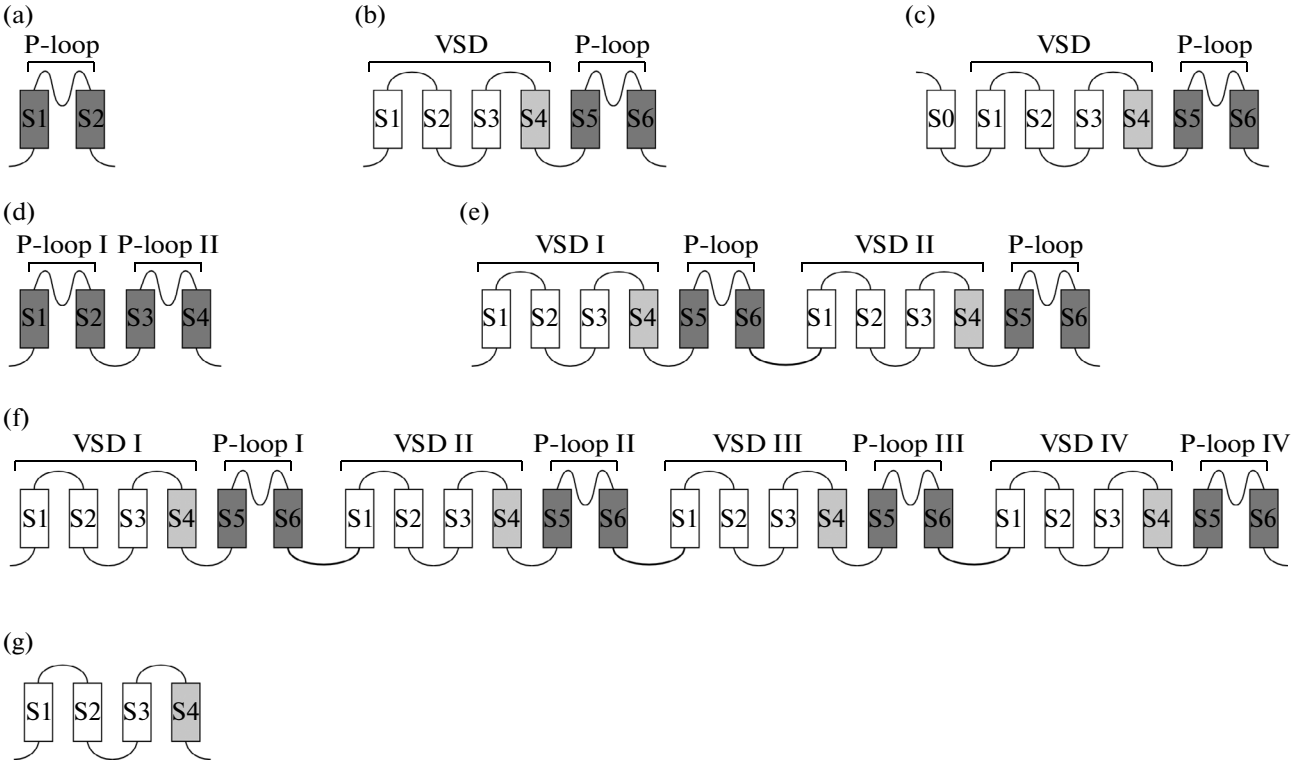

Fig. 1. Structural organization of ion channels of the voltage-gated cation channel superfamily: (a) inwardly rectifying potassium channel (K_{ir}), (b) voltage-gated potassium channel (K_v), EAG, HCN/CNG, and TRP channels, (c) calcium-activated potassium channel (K_{Ca}), (d) potassium leak channels (K_{2P}), (e) TPC channels, (f) voltage-gated calcium (Ca_v), sodium (Na_v), and NALCN channels, (g) voltage-gated proton channel (H_v).
P-loop—pore loop, *VSD*—voltage-sensitive domain, *S0–S6*—transmembrane segments, *I–IV*—domain numbers.

opment were observed in dinoflagellates *Noctiluca miliaris* (Eckert and Sibaoka, 1968; Oami et al., 1990).

We infer that the limited number of data on ion channels of dinoflagellates is related to the methodological obstacles that did not allow application of the patch-clamp technique to their cells until recently. At present, this method is the most powerful tool to study ion channel functioning (Sakmann and Neher, 2009). Patch-clamp involves the formation of a tight contact between a registering glass pipette and a cell membrane (Molleman, 2003). In case of intact dinoflagellates cells, the formation of a tight contact is impossible because of the complex armored cell covering of many species (Morrill and Loeblich, 1983; Pozdnyakov and Skarlato, 2012). In the previous work, we developed a method to obtain spheroplasts of armored dinoflagellates *Prorocentrum minimum* and for the first time recorded ion channels of these microorganisms at the single-channel level (Pozdnyakov et al., 2014). The diversity of ion channels described by the methods of electrophysiology can be further identified at the molecular level by the analysis of the *P. minimum* transcriptomes.

Most of the known ion channels of pro- and eukaryotes belong to the so-called voltage-gated cation channel superfamily. Since the identification of this superfamily is defined by homology of their amino acid sequences and channel domain structure, not all its members represent the channels that are indeed activated by shifts in the membrane potential. The name "voltage-gated cation channel superfamily" merely reflects the fact that the first described ion channels of this group were the true voltage-gated ion channels (Hille, 2001; Yu et al., 2005; Jegla et al., 2009).

Figure 1 shows the types of structural organization characteristic to the different members of the voltage-gated cation channel superfamily. Most members of the superfamily possess the common structural unit—two transmembrane segments with a pore loop (P-loop) between them (Fig. 1a-f). Multicellular organisms (Metazoa) have inwardly rectifying potassium channels (K_{ir}) that include only this structural unit (Fig. 1a) (Jegla et al., 2009).

A large group of channels, which includes several families, additionally bears a voltage-sensitive domain (VSD) that consists of four transmembrane segments S1-S4. The arginine/lysine-rich segment S4 functions as a voltage sensor in those channels that are activated by shifts in the membrane potential (Fig. 1b). Such domain organization is typical of the voltage-gated potassium channels (K_v), two families of the nucle-

otide-gated channels (ether-a-go-go-like channels—EAG and hyperpolarization-activated cyclic nucleotide-gated/cyclic nucleotide-gated channels—HCN/CNG), big family of transient receptor potential channels (TRP), and calcium-activated potassium channels (K_{Ca}) with the additional S0 segment (Fig. 1c). A domain comprising the segments S1-S6 forms an ion channel subunit that functions in a cellular membrane as a tetramer. C-terminal region of all these channels contains several extra domains that assist in homo- and heterotetramerization of ion channel subunits and in secondary-messenger binding (Hille, 2001; Yu et al., 2005; Jegla et al., 2009). Furthermore, animal cells have two-pore potassium channels (K_{2P}) which represent a doubled structure S1-P-loop-S2 homologous to the K_{ir} channels (Fig. 1d) (Jegla et al., 2009).

Multicellular animals also possess the so-called two-pore calcium channels (TPC) that have subunits consisting of two domains [S1–S6] (Fig. 1e) homologous to the K_v channels (Jegla et al., 2009). At the same time, the channels with the four-domain [S1-S6] structure also exist, e.g. voltage-gated calcium (Ca_v) and sodium (Na_v) channels (Hille, 2001), as well as voltage-independent sodium-leak channels (NALCN) (Yu et al., 2005; Jegla et al., 2009). Voltage-gated proton channels (H_v) represent a special group of channels because their subunits comprise only one domain [S1-S4] homologous to VSD (Fig. 1g) (DeCoursey, 2008; Jegla et al., 2009).

In the present work, we have analyzed the translated transcriptomes of *P. minimum* (strains CCMP1329 and CCMP2233) available in the database of Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP; http://data.imicrobe.us/project/view/104) and identified representatives of the various ion channel families within the large voltage-gated cation channel superfamily in this species.

MATERIALS AND METHODS

Transcriptome databases. In this work, we used the translated transcriptomes of two *P. minimum* strains (Prorocentrum-minimum-CCMP1329 and Prorocentrum-minimum-CCMP2233) available in the database of Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP; http://data.imicrobe.us/project/view/104, Combined Assemblies; Keeling et al., 2014).

Local BLAST search was performed by means of the BioEdit 7.2.5 software (Hall, 1999) with BLOSUM62 matrix (E-value < 10^{-10}). Human amino acid sequences from the NCBI protein database (http://www.ncbi.nlm.nih.gov/protein/) were used as queries.

Multiple alignment of amino acid sequences was carried out using the algorithm MAFFT 7 (Katoh and Standley, 2013). The obtained multiple alignments were further analyzed by means of BioEdit 7.2.5 (Hall, 1999) and Unipro UGENE (Okonechnikov et al., 2012) software. At present, there are about 10000 amino acid sequences of the voltage-gated cation channel superfamily in the NCBI database. We used amino acid sequences of metazoan ion channels (*Anopheles darling, Apis mellifera, Drosophila melanogaster*, *Homo sapiens*) in multiple alignments. Moreover, in order to illustrate a wide spread of the certain ion channel families, we used the sequences of plants (*Arabidopsis thaliana*), green algae (*Chlamydomonas reinhardtii*), ciliates (*Tetrahymena thermophila*), dinoflagellates (*Karlodinium veneficum*), and bacteria (*Burkholderia cenocepacia* and *Escherichia coli*).

Identity of two homologous sequences was calculated by means of SIAS online tool (http://imed.med.ucm.es/Tools/sias.html) as a percent ratio of the number of identical amino acid residues in the alignment to the length of the shortest sequence.

RESULTS

Analysis of the translated transcriptomes of the dinoflagellate *P. minimum* revealed amino acid sequences sharing homology with the majority of known ion channel types of the voltage-gated cation channel superfamily (see table).

In *P. minimum*, homologues of the K_{ir} channels possess a signature GYG in the locus of P-loop, which is characteristic to most potassium channels of other living organisms (Fig. 2a). Identity of amino acid sequences of the K_{ir} channels of *H. sapiens* (445 a. a.) and *P. minimum* (690 a. a.) was 27% (445 a. a.—length of the aligned fragment, hereinafter).

Single-domain channels. The *P. minimum* K_v homologues, also bearing the signature GYG, were present in both analyzed transcriptomes as two types of sequences with the lengths 400–600 a. a. and 1000 a. a. Sequences of the former group had a typical structure of the K_v channels (Fig. 1b). Sequences of the latter group represented the K_v channels with two homologous domains [S1–S6] (100 % identity, 570 a. a.) (Fig. 1e). The sequences of both the first group and each domain of the second group show 24–25% similarity with the human K_v channels.It is important to note that the presence of the K_v channel sequences containing two structural domains [S1-S6] in the *P. minimum* transcriptomes may be a result of either transcriptome assembling mistake or real duplication of the ancestral gene.

Sequences of the K_{Ca} channels of *P. minimum* (1100–1200 a. a.) which are homologous to the large conductance calcium-activated potassium channel (BK) of Metazoa also contain the GYG signature (Fig. 3). Similar to the BK channels of animals, the C-terminal region of K_{Ca} of *P. minimum* has an RCK-domain involved in the interaction of the channel with calcium ions. The K_{Ca} channel of *P. minimum* (1204 a. a.) shows 22% identity (1204 a. a.) to the BK

Translated sequences of the ion channel genes of various families revealed in the transcriptomes of Prorocentrum-minimum-CCMP1329 and Prorocentrum-minimum-CCMP2233

Ion channel family	Prorocentrum-minimum-CCMP1329		Prorocentrum-minimum-CCMP2233	
	number of the sequence	length, a.a.	number of the sequence	length, a.a.
K_{ir}	Not identified	–	35921_1	690
K_v (1)	51627_1	433	124306_1	365
K_v (2)	906_1	973	20439_1	985
K_{Ca}	52318_1	1118	2963_1	1204
EAG	263403_1	820	11858_1	814
HCN/CNG (1)	33715_1	744	35067_1	551
HCN/CNG (2)	258896_1	1163	14899_1	1363
K_{2P}	Not identified	–	Not identified	–
TRPV	42031_1	875	15802_1	904
TRPP	262008_1	1141	17215_1	1175
TPC	39996_1	827	16420_1	773
Ca^{2+}/Na_v^+	259712_1	1533	40145_1	2087
NALCN	Not identified	–	Not identified	–
H_v	146776_1	288	128_1	291

Data from MMETSP database analysis (http://data.imicrobe.us/project/view/104, Combined Assemblies).

channel of *H. sapiens* (1236 a. a.); the length of its RCK-domain is 180 a. a.

In the transctiptomes of *P. minimum*, the homologues of the nucleotide-gated channels (EAG and HCN/CNG) were found. All these channels bear a cyclic nucleotide-binding domain in their C-terminal regions (CNBD) (Craven, Zagotta, 2006; Vandenberg et al., 2012). Whereas EAG potassium channels of multicellular animals possess the signature GFG, their homologues sequences in *P. minimum* display the typical for most other potassium channels signature variant GYG (Fig. 4). EAG sequences of *P. minimum* also contain a 180 a. a. CNBD-domain. Identity of the EAG channel of *P. minimum* (ca. 800 a. a.) and EAG channel of *H. sapiens* (1159 a. a.) was 23% (820 a. a.).

The family HCN/CNG of both animals and their sister lineage, choanoflagellates, consists of two very close sub-families HCN and CNG which include non-selective cation channels. Interestingly, the HCN channels contain the GYG signature, which is probably responsible for their higher selectivity for potassium ions as compared to the CNG channels lacking such a signature (Craven and Zagotta, 2006). The *P. minimum* homologues of the HCN/CNG family channels possess the GYG signature (Fig. 4), which may indicate some selectivity of these channels for potassium ions. The CNBD domain has a length of 130 a. a. Similar to the homologues of K_v channels, the sequences homologues to the channels HCN and CNG of *P. minimum* can be divided into two groups: (1) sequences with one structural domain [S1–S6] (500–700 a. a.) (Fig. 1b), and sequences containing two domains (1100–1300 a. a.) (Fig. 1e). This also can be a result of a transcriptome assembling mistake or real duplication of the ancestral gene. Identity of the HCN and CNG sequences of *H. sapiens* (600–900 a. a.) and their homologues in *P. minimum* was 18–23% (551–744 a. a.).

TRP channels represent a big family of nonselective cation channels characterized by diverse mechanisms of activation. There are 8 sub-families of the TRP channels in animals—TRPA, TRPC, TRPP, TRPV, TRPN, TRPM, TRPML, and TRPVL (Jegla et al., 2009). Domain structure of these channels is similar to that in the K_v channel (Fig 1b). However, a domain homologous to VSD in K_v does not function as a voltage sensor. Moreover, the channels of several subfamilies (TRPA, TRPC, TRPV, and TRPN) contain 2–20 ankyrin repeats (Ank) in their N-terminus (Li et al., 2011). In the present work, we found sequences (870–900 a. a.) homologues to TRPV channels of Metazoa, which bear the Ank-domain of about 150 a. a. length (Fig. 5). The revealed sequences shared 23 % (729 a. a.) identity with the TRPV5 channel of *H. sapiens* (729 a. a.). Moreover, the *P. minimum* transcriptomes also contained homologues of TRPP channels (1100 a. a.) without Ank-repeats.

Two- and four-domain channels. Analysis of *P. minimum* transcriptomes revealed the TPC channel homologues (770–830 a. a.) with 22% (827 a. a.) identity to the human TPC channel (888 a. a.) (Fig. 6).

Furthermore, sequences of 1500–2000 a. a. length homologous to the animal Ca_v and N_v channels were

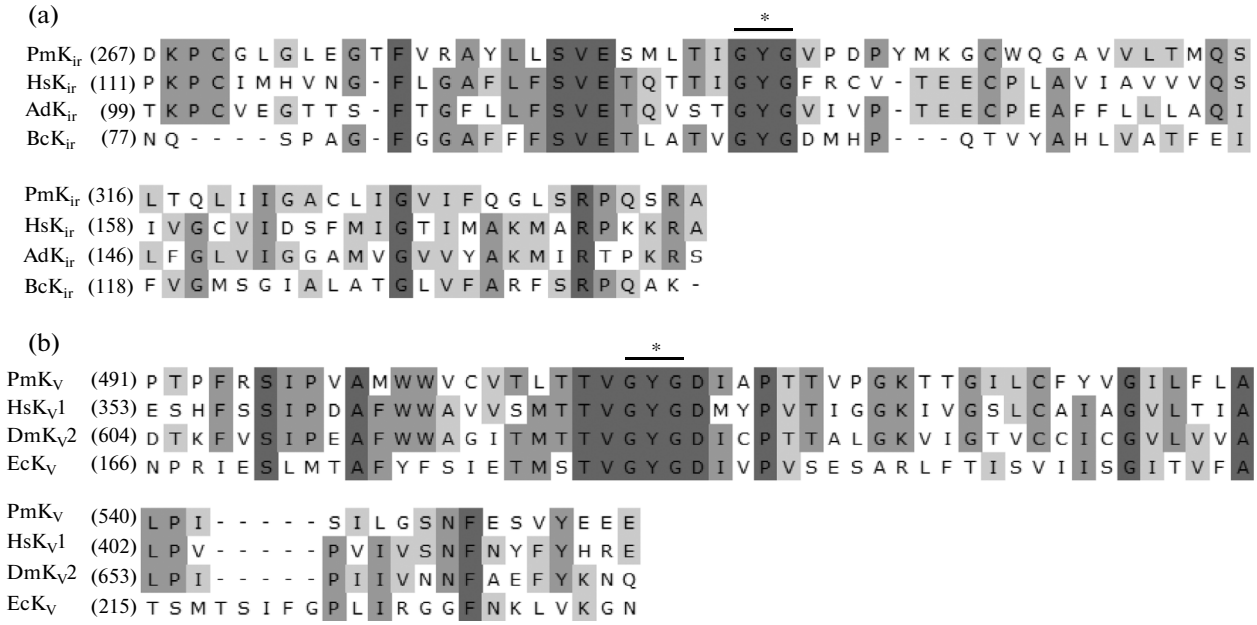

Fig. 2. The outcome of multiple alignment of homologous regions in amino acid sequences of (a) inwardly rectifying potassium channels (K_{ir}), and (b) voltage-gated potassium channels (K_v). *AdK_{ir}—Anopheles darling* (NCBI: ETN66688.1), *BcK_{ir}—Burkholderia cenocepacia* (NCBI: EPZ91042.1), *DmK_v—Drosophila melanogaster* (NCBI: AAC33365.1), *EcK_v—Escherichia coli* (NCBI: CAR12756.1), *HsK_{ir}—Homo sapiens* (NCBI: AAA19962.1), *HsK_v—H. sapiens* (NCBI: NP_000208.2), *PmK_{ir}—Prorocentrum minimum* (Prorocentrum-minimum-CCMP2233, MMETSP: 35921_1), *PmK_v—P. minimum* (Prorocentrum-minimum-CCMP1329, MMETSP: 906_1). Star—GYG signature (here and in Figs. 3, 4). Number in parentheses—order number of the first amino acid residue in a row (Figs. 2–8). Intensity of color reflects a degree of identity (Figs. 2–8).

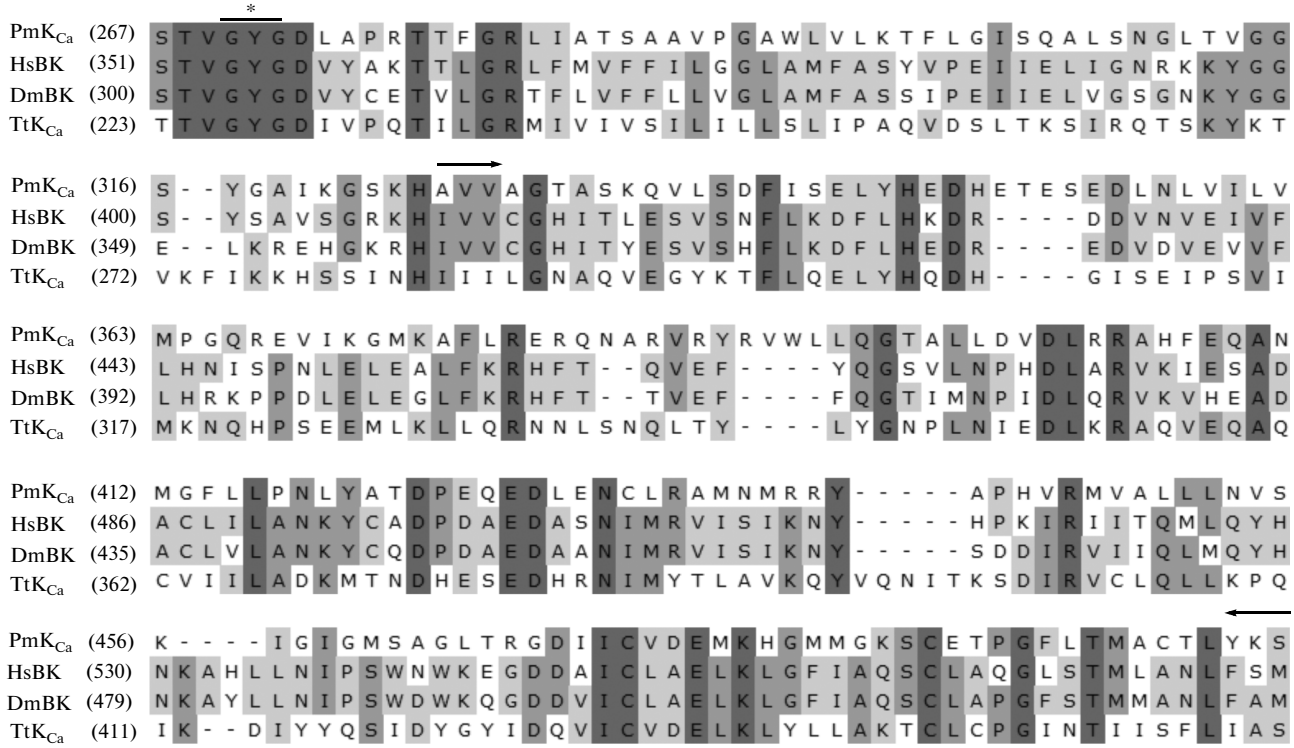

Fig. 3. The outcome of multiple alignment of homologous regions in amino acid sequences of calcium-activated potassium channels (K_{Ca}) in different species. *DmBK—Drosophila melanogaster* (NCBI: Q03720.3), *HsBK—Homo sapiens* (NCBI: Q12791.2), *PmK_{Ca}—Prorocentrum minimum* (Prorocentrum-minimum-CCMP2233, MMETSP: 2963_1), *TtK_{Ca}—Tetrahymena thermophila* (NCBI: EAR88631.2). Arrows indicate RCK domain.

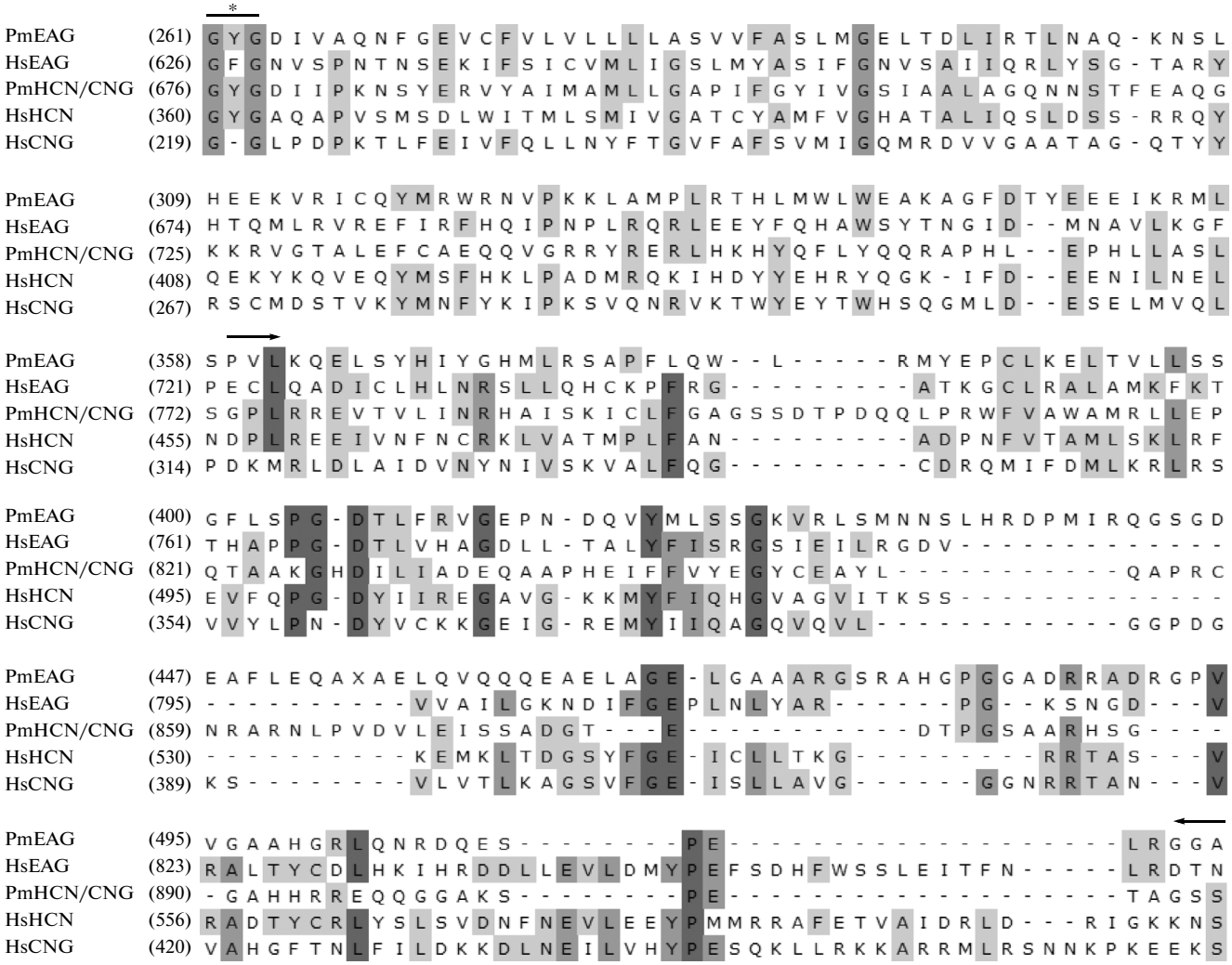

Fig. 4. The outcome of multiple alignment of homologous regions in amino acid sequences of channels of EAG and HCN/CNG families in the dinoflagellate *Prorocentrum minimum* and human. *HsCNG—Homo sapiens* (NCBI: AAA65619.1), *HsEAG—Homo sapiens* (NCBI: Q12809.1), *HsHCN—H. sapiens* (NCBI: NP_066550.2), *PmEAG—Prorocentrum minimum* (MMETSP, Prorocentrum-minimum-CCMP1329: 263403_1), *PmHCN/CNG—P. minimum* (MMETSP, Prorocentrum-minimum-CCMP1329: 258896_1). Arrows indicate CNBD domain.

found. These sequences can be assigned to the members of the Ca_v/Na_v channels family and possess typical four-domain organization. Identity of the Ca_v/Na_v channels of *P. minimum* and human $Na_v1.1$ (2009 a. a.) is 22% (2009 a. a.). A putative selectivity filter, consisting of four amino acid residues from P-loops of four domains, has E/E/E/E structure. Such selectivity filters are common to the mammalian L-type Ca_v channels. Comparison of these *P. minimum* sequences with the human L-type Ca_v channel (1977 a. a.) revealed 21% identity (1977 a. a) (Fig. 7a–7d). It should be noted that BLASTP search of the NALCN channel sequences against the translated transcriptomes of *P. minimum* reveals the same sequences as the search with the Ca_v and Na_v channels as querries. However, the primary structure of the transmembrane S4 segment of each domain in *P. minimum* was rather similar to that of Ca_v and Na_v channels (Fig. 7e).

Voltage-gated proton channels (H_v) are the distinct group of the voltage-gated cation channel superfamily, since they represent one domain with the S1-S4 transmembrane segments homologous to the VSD domain of K_v channels (Smith et al., 2011) (Fig 1g). The revealed sequences (280–290 a. a.) shared 20 % identity (273 a. a.) with H_v channels of *H. sapiens* (273 a. a.) and 26% identity (248 a. a.) with H_v of the dinoflagellate *Karlodinium veneficum* (248 a. a.) discovered by Smith and colleges (Smith et al., 2011) (Fig. 8).

DISCUSSION

The most complete information concerning the presence of various ion channels in a certain species,

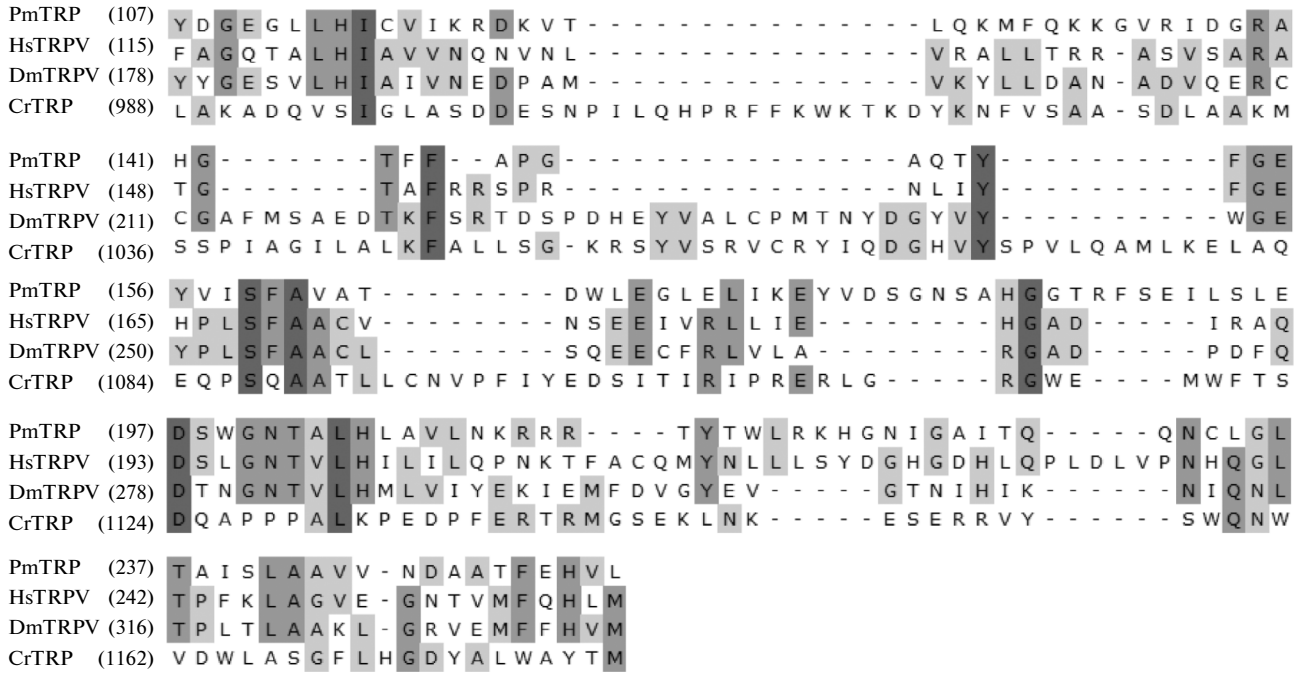

Fig. 5. The outcome of multiple alignment of homologous regions in amino acid sequences of ankyrin domains in TRP channels in different organisms. *CrTRP—Chlamydomonas reinhardtii* (NCBI: XP_001694631), *DmTRPV—Drosophila melanogaster* (NCBI: AAP57097.1), *HsTRPV—Homo sapiens* (NCBI: Q9NQA5.2), *PmTRP—Prorocentrum minimum* (MMETSP, Prorocentrum-minimum-CCMP2233: 15802_1).

as well as the knowledge of the evolution of these channels, can be obtained by the analysis of fully sequenced genomes of the respective organisms. However, the genome of *P. minimum* has not been sequenced yet because dinoflagellates possess very big genomes that often exceed the human genome in size and thus are difficult to decipher (Hackett et al., 2004). Therefore, the best way to search genes in these organisms is to screen their transcriptomes. In the present work, we performed screening of two *P. minimum* transcriptomes and found representatives of many ion channel families of the voltage-gated cation channel superfamily.

Although the sequences revealed in the transcriptomes of *P. minimum* are less than 30% identical to the ion channel sequences of Metazoa (*H. sapiens*), they have all structural motifs characteristic of the respective ion channel families. It should be noted that in case of all found homologues, E-value describing a degree of homology was less than 10^{-10}, which is sufficient to attribute the sequences of *P. minimum* to the members of the respective ion channel families in animals (Pearson, 2013).

At present, published information on the ion channels in dinoflagellates is scarce, and it was mainly obtained by means of electrophysiological studies. In particular, it was shown that dinoflagellates *Noctiluca miliaris* have voltage-activated Na^+, H^+ and Cl^- currents (Eckert and Sibaoka, 1968; Oami et al., 1990), and dinoflagellates *K. veneficum* possess H_v channels (Smith et al., 2011). In turn, the analysis of transcriptomes demonstrated that there are representatives of at least ten ion channel families in *P. minimum* (including two TRP channel subfamilies).

Potassium channels K_{ir}, K_v, K_{Ca} and K_{2P} participate in the regulation of a cellular membrane potential (Hille, 2001; Zefirov, Sitdikova, 2010). The fact that all these channels, except K_{2P}, have been found in the transcriptomes of *P. minimum* indicates that a similar membrane potential control system may exist in dinoflagellates. Moreover, the lack of the sequences homologous to K_{2P} in the transcriptomes of dinoflagellates *P. minimum* does not mean that these genes are lacking in their genome.

In the present work, it was shown that the genes of the TRP, EAG and HCN/CNG channels are present in the transcriptomes of *P. minimum*. The TRP channels of *P. minimum* are represented by at least two subfamilies, TRPV and TRPP, which is consistent with the publications about the earliest divergence of these two TRP channel groups (Cai, Clapham, 2012). The channels of TRP, EAG and HCN/CNG families are mainly involved in the signal transduction in cells of animals and protists. For instance, ciliates *Paramecium tetraurelia* express complex behavioral reactions (escape response and avoiding reaction), and most of their potassium channel genes encode the channels activated by cyclic nucleotides (Martinac et al., 2008). At the same time, the genome of green algae *Chlamydomonas reinhardtii* contains six to eight representa-

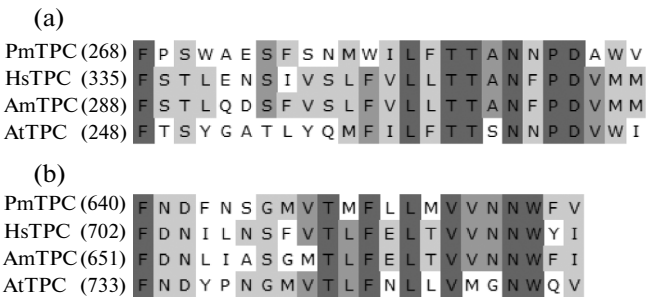

Fig. 6. The outcome of multiple alignment of homologous regions in amino acid sequences of the first (a) and the second (b) domains of TPC channels in different organisms. *AmTPC—Apis mellifera* (NCBI: NP_001201833.1), *AtTPC—Arabidopsis thaliana* (NCBI: NP_567258.1), *HsTPC—Homo sapiens* (NCBI: NP_001137291.1), *PmTPC—Prorocentrum minimum* (MMETSP, Prorocentrum-minimum-CCMP2233: 16420_1).

tives of the TRP channel family that play an important role in the behavior of these organisms, e.g. in mechanoreception (Huang et al., 2007; Fujiu et al., 2011; Arias-Darraz et al., 2015).

Dinoflagellates also express various behavioral reactions, i.e. geo-, chemo- and phototaxis, diel vertical migration in the water column and stress-induced ecdysis (Kamykowski et al., 1998; Pozdnyakov and Skarlato, 2012). Although molecular mechanisms of these reactions are poorly studied in dinoflagellates, we assume that the TRP, EAG and HCN/CNG channels are important in their development, since these channels function in the perception of external stimuli.

At present, it is an accepted viewpoint that two- and four-domain ion channels are a result of sequential duplications of a gene that initially encoded a cation channel showing typical for K_v channels single-domain structure. The first round of the ancestral gene duplication resulted in the origin of ion channels that contained two homologues domains in a single sequence. Each domain consisted of the segments S1–S4, which form VSD, and segments S5–S6 with the P-loop between them (Fig. 1e). The two-domain calcium TPC channels are thought to be an example of such gene duplication. These channels were revealed in the membranes of intracellular vacuoles in animals and plants. Moreover, they were identified in the genomes of some protists (Jegla et al., 2009; Cai, 2012). Our findings on the TPC channels in the transcriptomes of *P. minimum* conform to the hypothesis on the putative gene duplication at the earliest stages of eukaryotic evolution, since the mentioned groups of organisms have a common ancestor in the very root of the global eukaryotic tree (Adl et al., 2012). Remarkably, the time of the next gene duplication that had led to the appearance of four-domain voltage-gated channels Ca_v, Na_v, and NALCN has been under discussion for a long time (Cai, 2012; Liebeskind et al., 2012).

It was suggested that appearance of the four-domain Na_v channels was related to the origin of the nervous system in Metazoa, since fungi and plants lack the respective genes in their genomes (Hille, 2001). However, the latest genomic studies on choanoflagellates and apusozoans disagree with this hypothesis. The investigations proved that appearance of the four-domain Na_v channels occurred before the origin of multicellularity (Liebeskind et al., 2011; Cai, 2012).

Since the amino acid sequences of Ca_v and Na_v channels and their *P. minimum* homologues possess equal degree of similarity between each other, the latter can be identified neither as Ca_v nor as N_{av} at the present stage of knowledge. On the one hand, our data on the presence of the Ca_v/Na_v family in dinoflagel-

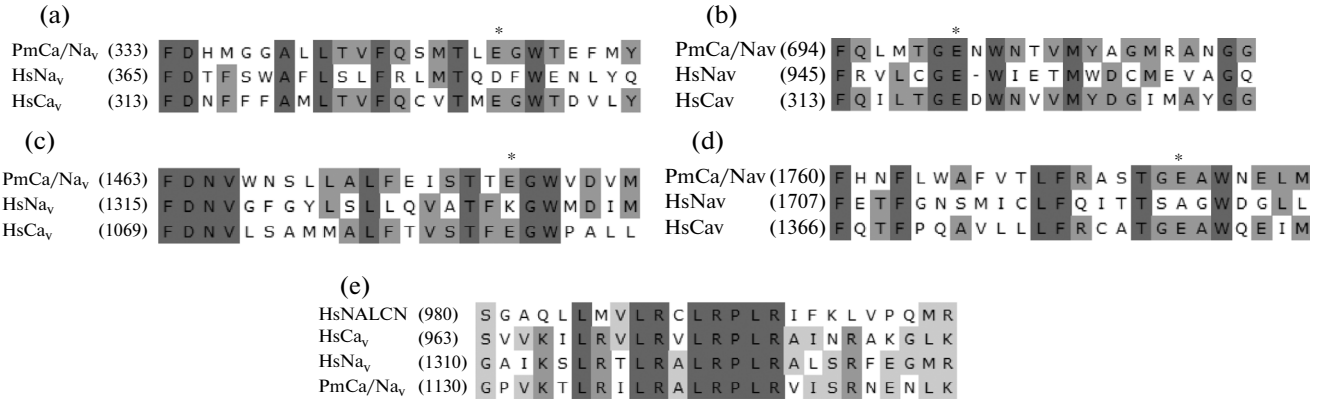

Fig. 7. The outcome of multiple alignment of homologous regions of pore loop (a-d) and segment S4 in domain III (e) of amino acid sequences in four-domain voltage-gated calcium (Ca_v), sodium (Na_v), and NALCN channels in human and Ca_v/Na_v channels in *Prorocentrum minimum*. *HsNALCN—Homo sapiens* (NCBI: NP_443099.1), *HsCa$_v$—H. sapiens* (NCBI: AAF15290.1), *HsNa$_v$—H. sapiens* (NCBI: NP_001189364.1), *PmCa/Na$_v$—Prorocentrum minimum* (MMETSP, Prorocentrum-minimum-CCMP2233: 40145_1). Stars—amino acid residues forming a selectivity filter.

lates conform to the electrophysiological data on the voltage-activated sodium current during propagation of the action potential in *N. miliaris* (Oami et al., 1990). On the other hand, the homologues of these channels possess the selectivity filter E/E/E/E that is typical for the Ca_v channels (Liebeskind et al, 2011), which may reflect calcium selectivity of the revealed channels. However, it should be noted that although the single-domain Na_v channels of bacteria contain a selectivity filter E/E/E/E, they still show selectivity for sodium, but not for calcium ions (Liebeskind et al, 2011). Thus, an in-depth structural and phylogenetic analysis is required to unambiguously attribute Ca_v/Na_v channels of *P. minimum* to the Ca_v or Na_v group. Nevertheless, already now we can suggest that Ca_v/Na_v channels of *P. minimum* participate in the processes requiring the action potential, such as control of flagella beating.

The presence of the H_v channels in dinoflagellates was first assumed during the investigation of bioelectrical control of bioluminescence in *N. miliaris* (Eckert and Sibaoka, 1968). The authors suggested that luminescence was triggered by a voltage-dependent entry of protons H^+ into lumen of the special membrane organelles (scintillones) containing the enzyme luciferase. In 2011, the H_v channel gene of the nonluminescent dinoflagellate *K. veneficum* was identified and cloned (Smith et al., 2011). *P. minimum* is not capable of luminescence either, but its H_v channels may play a role in the pH regulation of various vesicular organelles, as it occurs in luminescent dinoflagellates and coccolithophorids (Eckert and Sibaoka, 1968; Taylor et al., 2011).

Ion channels are tightly involved in all physiological processes in a cell, and dinoflagellates are not seemingly an exception in this respect. Studies of ion channels in dinoflagellates are necessary to understand many aspects of physiology of these ecologically important organisms, as well as the evolution of ion channels in general. Thus, the analysis of transcriptomic databases is of particular importance because it demonstrates the existing diversity of these transmembrane proteins and facilitates unraveling of their evolution. In the present work, we identified the following homologues of ion channels in the *P. minimum* transcriptome: 1—inwardly rectifying potassium channels, 2—voltage-gated potassium channels, 3—calcium-activated potassium channels, 4—cyclic nucleotide-gated channels, 5—TRPV and TRPP channels, 6—two-pore calcium channels TPC, 7—voltage-gated sodium and calcium channels, and 8—voltage-gated proton channels. Nevertheless, it is evident that physiological functions of each ion channel group must be studied by the methods of electrophysiology.

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Fig. 8. The outcome of multiple alignment of homologous regions of S4 segment of amino acid sequences in voltage-gated proton channels (H_v) of dinoflagellates and human. *HsH$_v$—Homo sapiens* (NCBI: NP_001035196.1), *KvH$_v$—Karlodinium veneficum* (NCBI: AEQ59286.1), *PmH$_v$—Prorocentrum minimum* (MMETSP, Prorocentrum-minimum-CCMP2233: 128_1).

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