Journal of Molecular Evolution (2024) 92:217–257
https://doi.org/10.1007/s00239-024-10168-x

ORIGINAL ARTICLE

Conservation of Protein Kinase A Substrates in the Cnidarian Coral Spermatozoa Among Animals and Their Molecular Evolution

Masaya Morita[1] · Nozomi Hanahara[1,2] · Mariko M. Teramoto[1] · Ariyo Imanuel Tarigan[1]

Received: 10 January 2024 / Accepted: 26 March 2024 / Published online: 25 April 2024

Abstract

The coral *Acropora* spp., known for its reef-building abilities, is a simultaneous hermaphroditic broadcast spawning species. *Acropora* spp. release gametes into seawater, activating sperm motility. This activation is mediated by adenylyl cyclase (AC) and protein kinase A (PKA). Notably, membrane-permeable cAMP (8-bromo-cAMP) promotes sperm motility activation of *Acropora florida*. While the signal transduction for PKA-dependent motility activation is highly conserved among animals, the downstream signaling of PKA remains unclear. In this study, we used mass spectrometry (MS) analyses to identify sperm proteins in the coral *Acropora digitifera*, as well as the serine/threonine residues of potential PKA substrates, and then, we investigated the conservation of these proteins from corals to vertebrates. We identified 148 sperm proteins of *A. digitifera* with typical PKA recognition motifs, namely RRXT and RRXS. We subsequently used ORTHOSCOPE to screen for orthologs encoding these 148 proteins from corals to vertebrates. Among the isolated orthologs, we identified positive selection in 48 protein-encoding genes from 18 *Acropora* spp. Subsequently, we compared the conservation rates of the PKA phosphorylation motif residues between the orthologs under positive and purifying selections. Notably, the serine residues of the orthologs under positive selection were more conserved. Therefore, adaptive evolution might have occurred in the orthologs of PKA substrate candidates from corals to vertebrates, accompanied by phosphorylation residue conservation. Collectively, our findings suggest that while PKA signal transduction, including substrates in sperm, may have been conserved, the substrates may have evolved to adapt to diverse fertilization conditions, such as synchronous broadcast spawning.

Keywords Protein kinase A · Sperm proteins · Codon evolution · Coral *Acropora* · Conservation of signal transduction

Introduction

Sperm flagellar motility is crucial for sexual reproduction, and the interaction between sperm and eggs is a prerequisite in various eukaryotes. Cnidarians, as phylogenetically early branching animals, exhibit diverse sexual reproduction, ranging from gonochoric to hermaphroditic, even within coral species (Siebert and Juliano 2017). For example, the reef-building coral *Acropora* is hermaphroditic (Baird et al. 2009), whereas mushroom corals, such as *Fungia* and *Ctenactis*, are gonochoric (Baird et al. 2009; Loya and Sakai 2008). Other cnidarians also exhibit diverse sexual modes; reproductive modes and regulatory mechanisms governing sperm flagellar motility leading to fertilization show diversity among cnidarians (Glass et al. 2023; Speer et al. 2021). However, the downstream mechanisms of this system require further exploration.

Upon activation of flagellar motility in the hermaphroditic *Montipora capitata*, a species of the *Acropora* family, and gonochoric *Astrangia poculata* corals, soluble adenylyl cyclase (sAC) produces cAMP, involving cAMP-dependent protein kinase A (PKA) (Glass et al. 2023; Speer et al. 2021). Notably, the Ac-PKA signal transduction is a highly conserved pathway for sperm flagellar motility activation in eukaryotes, contributing to activation in various animals such as sea urchins (Loza-Huerta et al. 2013; Nakajima et al. 2005), ascidians (Nomura et al. 2000; Shiba and Inaba 2014), teleosts (Inaba et al. 1999, 1998), and mammals

Handling editor: **Willie Swanson**.

✉ Masaya Morita
morita@lab.u-ryukyu.ac.jp

[1] Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Motobu, Okinawa 905-0227, Japan

[2] Okinawa Churahima Foundation, 888 Ishikawa, Motobu, Okinawa 905-0206, Japan

(Baro et al. 2020; Vyklicka and Lishko 2020). Moreover, the sequences of the catalytic subunit of PKA are highly conserved (Soberg et al. 2013). However, the downstream phosphorylation via PKA remains unclear.

Despite the conservation of Ac-PKA signal transduction, the identification of PKA substrates is imperative. In salmon sperm, PKA phosphorylates the motor protein dynein light chain during flagellar motility initiation (Inaba et al. 1999). Other PKA-dependent protein kinase substrates include A-kinase anchoring protein 4 in human spermatozoa (Su et al. 2010), tyrosine kinase (Alvau et al. 2016), phosphatase, ion channels, and GTP binding proteins (Fujita et al. 2000; Leclerc and Kopf 1999). In sea urchins, several substrates have been identified, including ATP synthase, creatine kinase, NADH dehydrogenase, tubulin beta chain, and cAMP-dependent protein kinase type II regulatory subunit (PKA RII; Loza-Huerta et al. 2013).

In the evolution and speciation of animals, axoneme architecture has remained conserved (Inaba 2011), whereas flagellar morphology has changed. For example, mammalian sperm flagella possess a fibrous sheath (Turner 2006), contrasting with phylogenetically early branching animals such as corals, whose flagella consist of an axoneme and a plasma membrane without the accessory structure found in mammalian sperm (Gaino et al. 2008; Padilla-Gamiño et al. 2011; Steiner 1991). Consequently, the anchoring proteins mediating flagellar motility initiation and activation should also change during evolution.

The fertilization conditions in corals and other animals exhibit distinct characteristics. For example, fertilization can occur either internally or externally, even within coral species (Baird et al. 2009). Various ecological factors may have influenced the evolution of their motility features. For the broadcast spawning coral *Acropora*, sperm must locate eggs for fertilization after being released into the water. However, sperm is susceptible to dilution (Levitan and Petersen 1995; Yund 2000), and its quantity may be correlated with fertilization success (Kitanobo et al. 2022). From an ecological perspective, ejaculations by multiple colonies or males in other taxa can potentially lead to sperm competition, potentially influencing sperm evolution (Pizzari and Parker 2009). Additionally, the reproduction of corals is essential for coral reef maintenance (Knowlton 2001). However, their sexual reproduction, spawning, is limited to a few times a year (Baird et al. 2021a). Sperm initiate coral reproduction via mating with other conspecific eggs. Notably, the genetic diversity of the colonies releasing sperm and their sperm number denote the fertilization success and genetic diversity of the fertilized eggs in the ocean (Kitanobo et al. 2022). Moreover, ocean acidification and local stressors influence coral reproduction (Hagedorn et al. 2016), such as a decline in egg numbers (Leinbach et al. 2021). In the mating pathways, ocean acidification potentially suppresses the alkalinization of sperm intraflagellar region, which is essential for sAC activation for sperm flagellar motility initiation (Morita et al. 2010; Nakajima et al. 2005), resulting in lower fertilization success (Albright et al. 2010). Therefore, understanding sperm flagellar motility under PKA is crucial for coral reproduction. Nevertheless, comprehensive analyses of the identification of PKA substrates and their potential roles in motility diversity are yet to be conducted.

To address these gaps, we aimed to identify sperm proteins in the reef-building coral *Acropora digitifera*, identify potential PKA substrates from the sequence data, explore orthologs, and assess the sequence conservation of phosphorylation residues. We believe that our research holds implications for comprehending the adaptive evolution of fertilization-related proteins, highlighting potential adaptations to specific fertilization conditions, and offering valuable information for broader comparative studies in the field of reproductive biology.

Materials and Methods

Corals and Sperm Collection for Mass Spectrometry (MS) Analyses

The coral *A. digitifera* (Fig. 1a), *Acropora tenuis*, and *Acropora florida* were collected 7 days before spawning at Sesoko Island, Okinawa, Japan (26.629, 127.862), in May or June 2019, 2020, and 2022. All colonies were collected from shallow reefs at depths ranging 2–5 m. The collected colonies (five colonies each from *A. digitifera, A. florida*, and *A. tenuis*) were placed in a flowing water aquarium under natural conditions. The gamete setting was monitored from 20:30 to 21:00, and colonies ready to spawn (Fig. 1b) were transferred to seawater-filled 5 L buckets to collect gametes from each colony. Spawning commenced around 19:20 to 19:40 in *A. tenuis* and 21:40 to 22:30 in *A. digitifera* and *A. florida*, releasing gamete bundles. Collected gamete bundles were separated into sperm and eggs using a plankton mesh following the method described by Morita et al. (2006). The isolated sperms were counted using a Thoma cell counting chamber (Erma, Tokyo, Japan), and 40 mL of seawater (SW) containing 1×10^7 to 6×10^6 spermatozoa (cells/mL) were centrifuged at $12{,}000 \times g$ for 10 min at 4 °C to obtain pelleted sperm cells. Isolated sperm samples were stored at − 30 °C until MS analyses.

Effect of Potassium Ionophore and Membrane-Permeable cAMP on Sperm Motility Activation

The sperm of *A. florida* ($N = 4$) were suspended in artificial seawater (ASW) containing 8-bromo-cAMP to confirm the

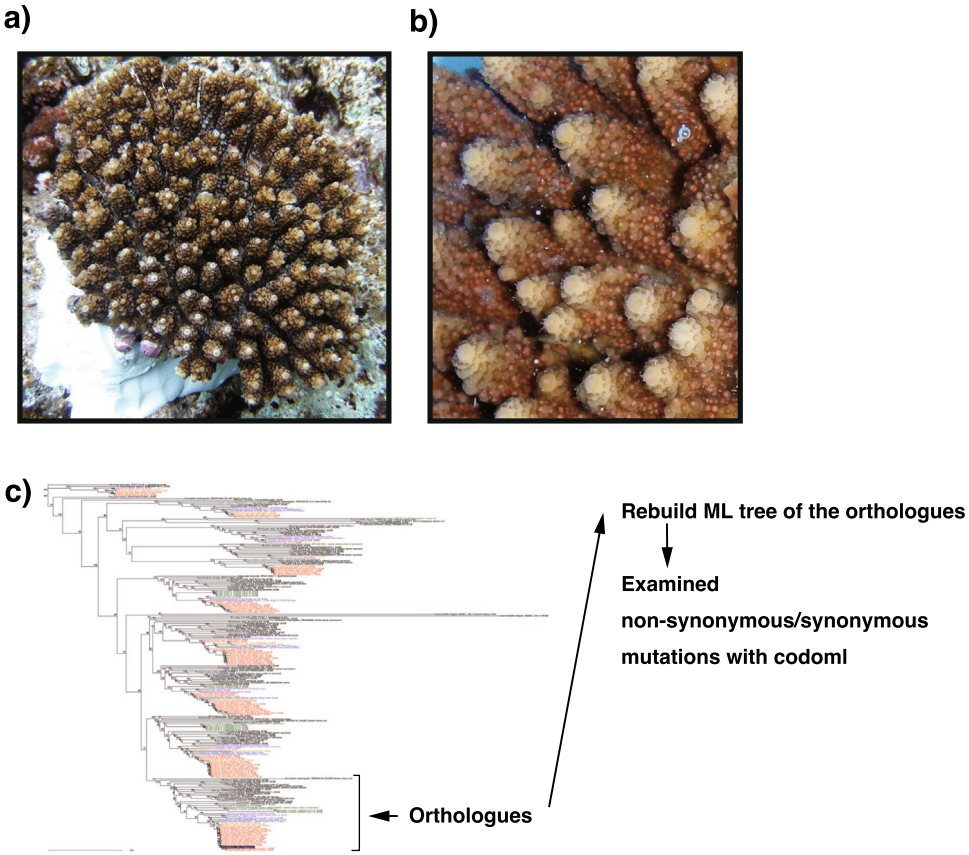

Fig. 1 Corals and analytical procedures. **a** Sperm protein analyses were conducted using the reef-building coral *Acropora digitifera* through mass analyses. **b** *Acropora*, which spawns once or a few times a year, exhibits the "setting" of gamete bundles on the surface of the colony. **c** Orthologs from the same clade of the identified protein were isolated

effect of cAMP on sperm motility activation. A total of 0.2 µL of sperm suspension was added to 100 µL of artificial seawater (ASW, 450 mM NaCl, 10 mM KCl, 9 mM $CaCl_2$, 30 mM $MgCl_2$, 16 mM $MgSO_4$, pH 8.0) containing 100 µM of 8-bromo-cAMP with DMSO (10 mM). We also examined the effect of potassium ionophore, valinomycin, which causes soluble adenylate kinase activation owing to membrane hyperpolarization (Izumi et al. 1999). The sperm of *A. tenuis* ($N=3$) were suspended in ASW containing 10 or 100 µM valinomycin in DMSO (1 mM or 10 mM, respectively). An activation solution containing NH_4Cl was used for the coral *Acropora* (0.5 M choline chloride, 20 mM NH_4Cl, and 10 mM HEPES–NaOH pH 8.0) as the positive control. The recording of the sperm was initiated from the beginning of the dilution, and the sperm showed motility roughly 30 s later. Subsequently, we recorded these sperm movements with a BlackMagic design connected with a CCD camera (Mintron, Taiwan) on the microscope (Nikon Optiphoto) under dark field illumination or phase contrast. The objective lens was × 10 with a × 10 relay lens. Sperm numbers were counted from the recordings (about 200 sperm in the field), and the motile percentage of the sperm was calculated. The swimming velocities were measured with three recordings converted into TIFF files for 1 s for 30 frames with Premier 2022 (Adobe). The converted TIFF files were opened with Image J, and the sperm head with color profiles was selected. Heads were then copied and pasted to a new file for 15 frames. The 15 framed pasted files indicate the trajectories of the sperm head, and we measured the length of each trajectory to calculate sperm movement for 0.5 s to calculate sperm swimming velocity.

Mass Spectrometry of Coral Sperm

Sperm proteins from *A. digitifera* ($N=1$) were subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS) analyses by Promega Japan and were identified using the NCBI GenBank dataset. All identified amino acid sequences were retrieved from the accession codes.

The eggs or sperm were treated with trypsin and subjected to nano-LC–MS analyses at Kazusa DNA Research Institute. The proteins were eluted with sonication in the elution solution [2 (w/v) % SDS and 100 mM Tris–HCl; pH 8.5]. The eluted protein concentrations were measured using the BCA assay and adjusted to 1 µg/µL with the elution solution. Tris(2-carboxyethyl)phosphine (TCEP) was added to 20 µg of proteins (the final concentration was 20 mM) and incubated for 10 min at 80 °C to cleave disulfide linkages. 2-Iodoacetamide (IAA) was then added for the alkylation of cysteine residues (final concentration of 30 mM) and

incubated for 30 min at room temperature around 25 °C in the dark. A mixture of Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophilic) and those of Hydrophobic was added in a 1:1 (v/v) ratio and washed with distilled water three times. These alkylated samples were adjusted to 15 µg solids/µL with SP3 beads (20 µL), to which 2.5 vol of 99.5% EtOH was added. The mixture was then incubated for 20 min at room temperature. The incubated beads were washed twice with 80% EtOH and then mixed with 100 µL of 50 mM Tris–HCl (pH 8.0). Peptide fragments from the proteins were obtained by adding 500 ng trypsin/Lys-C mix (Promega, Madison, WI, USA) into the beads and Tris base (50 mM Tris–HCl; pH 8.0), followed by incubation at 37 °C overnight. Then, 20 µL of 5% trifluoroacetic acid (TFA) was added to the trypsin-treated mixture and sonicated. The mixture was desalinized using reversed-phased spin columns (GL-TIP SDD; GL Science, Tokyo, Japan), and the desalinated samples were evaporated using the rotary evaporator. The dried samples were diluted with 2% acetonitrile (ACN) and 0.1% TFA and then sonicated to dissolve the dried peptides. The concentration of the peptides was measured using the BCA assay and adjusted to 200 ng/µL with 2% ACN and 0.1% TFA. These peptide samples were analyzed via LC–MS (nano-LC: UltiMate 3000 RSLCnano LC system; Thermo Fisher Scientific, Waltham, MA, USA) with MS (Orbitrap Exploris 480) in ESI positive mode. The peptides were sprayed from a column (120 mm length and 75 µm diameter) at 40 °C. The flow rates were 750 nL/min for the first 4 min, and then 200 nL/min for the remaining 40 min. The mobile phase consisted of (A) 0.1% formic acid and (B) 0.1% formic acid and 80% ACN. The mixture rates of the A and B mobile phases were as follows: starting from 3% B solution, increasing to 10% B at 10 min, then 1.375%/min, increasing up to 65% B at 44 min. MS analyses were performed in overlapping window DIA modes with successive analyses with the following four events: (1) full scanning (MS1), (2) isolation window 1 DIA (MS2), (3) full scanning (MS1), and (4) isolation window 2 DIA (MS2). The mass data were analyzed with scaffold DIA (Proteome software) with the protein database of *A. digitifera* from NCBI (34,278 entries). A spectral library was built with Prosit (https://www.proteomicsdb.org/prosit/) using the NCBI database of *A. digitifera*. The settings were as follows: Fragmentation: HCD, Precursor Tolerance: 10 ppm, Fragment tolerance: 10 ppm, Data acquisition type: Overlapping DIA, Digestion enzyme: Trypsin, Peptide Charge: 2 to 4, Max Missed Cleavages: 1, Fixed modification: Carbamidomethylation, Peptide false discovery rate (FDR): > 1%, and Protein FDR: > 1%.

The proteins were searched with scaffold DIA to identify them from the mass results. The data were shared at jPOSTrepo (announced ID JPST002975 PXD050472).

Searching for PKA Substrate

PKA substrates were isolated from candidate sequences with RRXS/T. The "grep" command in the terminal in Macintosh was used to isolate PKA substrate (grep "RR.S" or grep "RR.T") candidates containing RRXS, RRXT, or both. A total of 165 proteins with phosphorylation residues were isolated from over 2000 mass spectrometry-identified *A. digitifera* sperm proteins.

Comparison with the PKA Substrates of the Vertebrates

The identified PKA substrates were compared with the PhosphoSitePlus Substrate of Kinase database (https://maayanlab.cloud/Harmonizome/dataset/PhosphoSitePlus+Substrates+of+Kinases) (Hornbeck et al. 2004, 2015). A total of 327 protein kinase, cAMP-dependent (PRKCA) substrates were identified. The overlap between these substrates and the candidates of PKA substrates was then analyzed.

Searching Orthologs of the PKA Substrate

From the isolated 165 candidates of PKA substrates, the orthologs of these coding sequences (CDs) were screened using ORTHOSCOPE v.1.5.2 (Inoue and Satoh 2019) to confirm that the isolated candidates have orthologs among corals, cnidarians, and vertebrates (Fig. 1c; Supplementary Table 1). This search was performed using the *Acropora* site (https://orthoscope.jp/orthoscope/Acropora.html) with the default settings applied. Subsequently, one ortholog group was selected from the amino acid sequence FASTA files from ORTHOSCOPE (010_candidates_prot.txt) to search for phosphorylation residues and nucleotides among the FASTA files (010_candidates_nucl.txt) for codon evolution analyses (Fig. 1c). The file was named "**_orthologs." Among the 165 proteins, 17 proteins orthologs were not successfully isolated with ORTHOSCOPE. Subsequently, orthologs for the remaining 148 proteins were successfully isolated. The script was then executed to determine the number of operational taxonomic units (OTU). Of the 165 candidates, 148 protein orthologs were identified. Subsequently, the script named "pick_phosphorylation_sites.sh" was used to identify and count the number of genes with phosphorylation residues among orthologs and *Acropora* in this ortholog (i.e., "RRXT = 8/120 RRXS = 30/120, Acropora RRXT = 0/45, Acropora RRXS = 13/45"). In this output, RRXS = 30/120 indicates that 8 orthologs from corals to vertebrates have RRXT from 120 orthologs, and Acropora RRXS = 13/45 indicates that 13 orthologs in the coral *Acropora* have RRXS from 45 orthologs in the *Acropora*. The scripts are available at https://github.com/Masaya0606/Isolation-of-PKA-subrtate-in-the-coral-Acropora.git.

Molecular Evolutionary Analyses and Conservation of Phosphorylation Residues in the Orthologs

The molecular sequence evolution of the isolated orthologs was analyzed in terms of non-synonymous and synonymous mutations, and the conservation of phosphorylation residues among the orthologs was checked. As described earlier, the coding sequences of the orthologs were isolated from the nucleotide FASTA files from ORTHOSCOPE (010_candidates_nucl.txt). The isolated coding sequences were aligned using MAFFT, and the aligned files were converted to NEXUS format. The converted files were re-examined using Mesquite, and files containing low-alignment sequences were removed. Subsequently, the files were cleaned and transformed into PHYLIP format to build a maximum likelihood phylogenetic tree using RaxML-NG for subsequent codon evolution analyses. A phylogenetic tree was built with a maximum likelihood criterion using the GTR-gamma model, the bootstrap values of the tree file were removed for the following molecular evolutionary analyses of codons with CodeML analyses.

CodeML, from PAML, was used for molecular sequence evolution. A codon residue model was used to examine molecular evolution. Before the analyses, a phylogenetic tree was built with RaxML-NG using the GTR-gamma model from the aligned PHYLIP file, which was removed from the tree; the edited tree and PHYLIP file for building the tree were used for the CodeML analyses. The conserved sequences of phosphorylation residues (RRXT/RRXS) among the orthologs were examined using the grep command described earlier.

In the CodeML analyses, to examine the codons in the orthologs under positive selection, two models were set: neutral evolution and positive selection. In the analyses, model8a was used for the null hypothesis and model8 for the positive selection. The two models were run separately, using ML phylogenetic trees as tree files and PHYLIP files as nucleotide files. Subsequently, the likelihood ratio between model8 and model8a was calculated ($\Delta \ln L = 2 * (\text{model8a} - \text{model8})$), and the positive selection was supported when $\Delta \ln L > 2.68$ (Yang 1997). The branch non-synonymous/synonymous mutation rate of codons (dN/dS ratio) was obtained from the output file of model8.

Statistical Analyses

A Wilcoxon rank-sum test was performed for the conservation of phosphorylation sites and the dN/dS ratio obtained from code analyses. For the motility analyses, Tukey's HSD test was applied after the homogeneity of variance was examined via Levene's test. Kruskal–Wallis test was conducted for swimming velocity in 8-bromo-cAMP. These analyses were performed using R (R Core Team 2021), and the level of statistical significance was $P < 0.05$.

Results

Involvement of Membrane Potential and cAMP

We examined the effect of potassium ionophore valinomycin and membrane-permeable cAMP, 8bro-cAMP on the sperm motility activation in *A. tenuis* or *A. florida*. In the presence of valinomycin, sperm started their sperm motility (Fig. 2a, Tukey HSD, ASW as a negative control vs. valinomycin 10 µM 100 µM, NH_4Cl treatment $P < 0.001$) and swimming velocity was also significantly different from the NH_4Cl-induced motility activation sperm (Fig. 2b, Tukey HSD $P < 0.05$). 8bro-cAMP induced sperm motility activation in artificial sweater (ASW, Fig. 2c, Tukey HSD, NH_4Cl-induced motility activation as a control vs. 8-bromo-cAMP 10 µM or 100 µM, $P < 0.001$); however, the swimming velocity was not significantly different (Fig. 2d, Kruskal's test, $\chi^2 = 8$, df = 8, $P = 0.43$). This result indicates cAMP-related sperm motility activation in the coral *Acropora* spp.

Candidates of the PKA Substrates

We identified 165 sperm-constituent proteins containing RRXT or RRXS sequences among the deduced amino acid sequences (Table 1). These candidates included motor proteins, such as dynein, adenylate kinase, mitochondrial and axonemal proteins, and several uncharacterized proteins. Subsequently, we searched for orthologs of these candidates, successfully isolating 148 orthologs out of the initial 165. Ten candidates were specific to invertebrates, cnidarians, or corals, whereas orthologs for approximately 20 candidates could not be identified. The copy number of several candidates increased in the corals; we included these paralogs in one ortholog group for subsequent analyses.

Comparison with the PKA Substrates of the Vertebrates

We compared the overlap of the 327 PKA substrates in the PhosphoSitePlus Substrate of Kinase database (Supplementary Table 2). Among the 165 candidates, only 7 substrates coincided with the record (Table 2).

Identification of Phosphorylation Residues Among the Orthologs

Most *Acropora* species contained conserved phosphorylation residues, with threonine residues (RRXT) being more

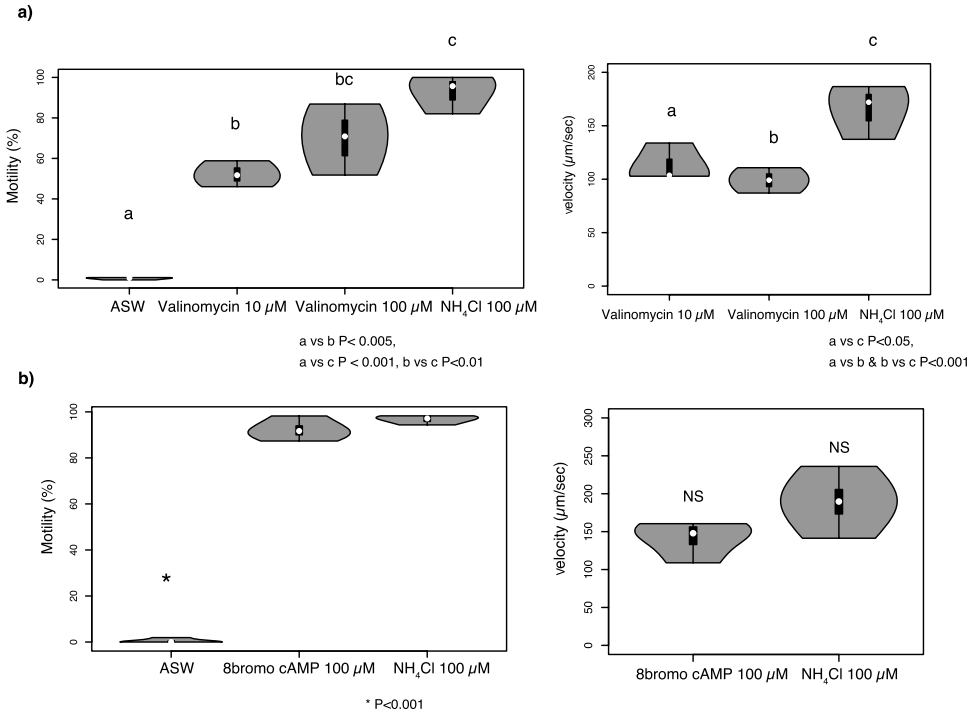

Fig. 2 Effect of potassium ionophore, valinomycin, and membrane-permeable cAMP, 8bro-cAMP, on coral sperm motility activation. **a** The effect of valinomycin, the potassium ionophore, on sperm motility activation, and **b** swimming velocity was examined. *Acropora tenuis* sperm were used for the analyses ($N=3$); **c** the effect of the membrane-permeable cAMP analog, 8-bromo-cAMP on motility and **d** swimming velocity was examined in *A. florida* sperm ($N=4$). Tukey's HSD test for motility and Kruskal–Wallis test were performed for swimming velocity. *Significant difference ($P<0.001$)

abundant than serine residues (RRXS). These residues are conserved in most dyneins, excluding T-complex protein 1 subunit epsilon-like (Protein no. 76-689 in Table 1). Additionally, phosphorylation residues were preserved in essential sperm components such as mitochondrial proteins (1081, 1583) and tubulin (347).

PKA substrate candidates displayed conservation across animals, with the identification of cnidarian-specific proteins. Notably, several uncharacterized proteins (Protein no. 52-346 in Table 1) were conserved among corals to *Homo sapiens*. Four E3 ubiquitin ligase candidates for PKA phosphorylation were identified, suggesting conserved ubiquitination from cnidarians to vertebrates and potential regulation by PKA phosphorylation. Eight cnidarian- or coral-specific, uncharacterized proteins were also identified. Seven proteins matched the database, with conserved serine or threonine of PKA phosphorylation residues.

Molecular Evolution of Candidate Proteins

We conducted molecular evolutionary analyses to compare the molecular evolutionary rates of codons and the conservation of phosphorylation residues. However, owing to the time-consuming nature of aligning orthologs across the animal kingdom, analysis was limited to cnidarian- or coral-specific orthologs. The codon site analysis with CodeML revealed that 48 out of the 148 orthologs were under positive selection in the coral *Acropora* (Table 1). This analysis provided information on whether specific codons were subject to positive selection, as indicated by a low d*N*/d*S* ratio in the open reading frames of many orthologs. Consistent with this prediction, the d*N*/d*S* ratio for the open reading frames of all isolated orthologs was low (Fig. 3a–d). Notably, the d*N*/d*S* ratio and the conservation of PKA phosphorylation residues in threonine among the orthologs were not negatively correlated (Fig. 3c, d).

Conversely, the conservation of serine residues in the orthologs under positive selection was significantly higher than that in those under purifying selection (Fig. 4a, b). However, the conservation of threonine residues did not exhibit the same trend (Fig. 4c, d). This trend was consistent across all orthologs. Therefore, 30% of PKA substrate candidates were under positive selection, whereas threonine residues remained conserved. Consequently, codon evolution did not impact the conservation of threonine phosphorylation, and serine residues were more conserved than those under purifying selection. The coding sequences of the seven proteins that matched with the database were subjected to strong purifying selection, whereas only one of them was subjected to positive selection (Table 2).

Discussion

In this study, we identified candidate PKA substrates in coral sperm and explored their diversity. Some candidates are specific to cnidarians or corals. Approximately one-third of these candidates underwent positive selection, indicating adaptive evolution. Proteins crucial for flagellar motility, including tubulin and dyneins, are conserved across

Table 1 Isolated PKA substrates in sperm proteins

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
2	XP_015752501.1	Common	PREDICTED: dynein heavy chain 12 axonemal-like [*Acropora digitifera*]	435	0.6042	0.1042	0.8000	0.0000					
3	XP_015768964.1	Common	PREDICTED: LOW QUALITY PROTEIN: dynein heavy chain 3 axonemal-like [*Acropora digitifera*]	442	0.6250	0.0417	0.6471	0.0000	−9180.159826	−9184.70081	9.081968	0.00258	0.0726*
4	XP_015770356.1	Common	PREDICTED: LOW QUALITY PROTEIN: hydrocephalus-inducing protein homolog [*Acropora digitifera*]	569	1.0000	1.0000	1.0000	1.0000	−22,503.79981	−22,511.95784	16.316052	0.00005	0.2606*
6	XP_015752737.1	Common	PREDICTED: LOW QUALITY PROTEIN: dynein heavy chain 7 axonemal-like [*Acropora digitifera*]	276	0.7885	0.6731	0.9375	0.9375	−759.619626	−806.165071	93.09089	0	17.9227*
7	XP_015761214.1	Common	PREDICTED: dynein heavy chain 1 axonemal-like partial [*Acropora digitifera*]	423	0.3793	0.2414	0.4000	0.3333	−11,087.34832	−11,092.45158	10.20652	0.0014	0.0745*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
10	XP_015756591.1	Common but many paralogues; Orthologues are found only in cnidarias	PREDICTED: LOW QUALITY PROTEIN: dynein heavy chain 8 axonemal-like [*Acropora digitifera*]	367	0.8824	0.4412	0.8947	0.5789	− 12,616.2862	− 12,719.64277	206.713138	0	1.0976*
15	XP_015764834.1	Orthologues were not found with Orthoscope (ND)	PREDICTED: adenylate kinase 9-like [*Acropora digitifera*]	203									
19	XP_015777523.1	ND	PREDICTED: uncharacterized protein LOC107355456 [*Acropora digitifera*]	203									
22	XP_015772855.1	ND	PREDICTED: uncharacterized protein LOC107351106 [*Acropora digitifera*]	763									
37	XP_015776505.1	ND	PREDICTED: uncharacterized protein LOC107354539 [*Acropora digitifera*]	195									

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
39	XP_015760595.1	Common	PREDICTED: cilia- and flagella-associated protein 61-like [*Acropora digitifera*]	148	0.1978	0.0769	0.3611	0.0000	− 2032.715592	− 2032.715592	0	1	0.1507
40	XP_015767939.1	Common	PREDICTED: E3 ubiquitin-protein ligase HUWE1-like [*Acropora digitifera*]	408	0.6885	0.7705	0.8889	0.9444	− 17,960.15937	− 17,960.15259	0	1	0.1017
47	XP_015771919.1	Up to Ciona	PREDICTED: glutamine-rich protein 2-like [*Acropora digitifera*]	91	0.7586	0.3793	0.8750	0.3750	− 5298.123133	− 5298.123394	0.000522	0.98177	0.2737
52	XP_015760851.1	Common	PREDICTED: kinesin-like protein KIF28P [*Acropora digitifera*]	108	0.3091	0.0727	0.8667	0.0000	− 3409.679968	− 3409.77091	0.181884	0.66976	0.3024
56	XP_015751383.1	Common	PREDICTED: uncharacterized protein LOC107331322 [*Acropora digitifera*]	108	0.6286	0.1286	0.7391	0.0435	− 2603.039206	− 2604.212544	2.346676	0.12555	0.2692
60	XP_015769367.1	Common *not higher veterbrates	PREDICTED: ATP-binding cassette sub-family A member 3-like [*Acropora digitifera*]	212	0.8356	0.0959	0.8421	0.0526	− 9817.53684	− 9875.022205	114.97073	0	1.3873*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
65	XP_015774896.1	Common	PREDICTED: LOW QUALITY PROTEIN: transitional endoplasmic reticulum ATPase-like [*Acropora digitifera*]	90	0.4688	0.0313	0.7647	0.0588	− 4426.611333	− 4427.82822	2.433774	0.11875	0.1146
70	XP_015775345.1	ND	PREDICTED: probable E3 ubiquitin-protein ligase HECTD4 [*Acropora digitifera*]	435									
75	XP_015762573.1	Common *up to *Homo sapiens*	PREDICTED: centrosomal protein of 290 kDa-like [*Acropora digitifera*]	280	0.4286	0.3571	0.6667	0.6667	− 8436.831752	− 8436.831752	0	1	0.1052
99	XP_015774601.1	Common *up to *Homo sapiens*	PREDICTED: uncharacterized protein LOC107352803 [*Acropora digitifera*]	104	0.4844	0.2031	1.0000	0.1333	− 5182.008123	− 5188.587214	13.158182	0.00029	0.2675*
103	XP_015769719.1	Common	PREDICTED: uncharacterized protein KIAA1109-like [*Acropora digitifera*]	530					− 15,843.21886	− 15,850.5319	14.626088	0.00013	0.2363*
106	XP_015761023.1	Common	PREDICTED: IQ and AAA domain-containing protein 1-like [*Acropora digitifera*]	92	0.3778	0.0667	1.0000	0.0000	− 3620.280456	− 3654.396099	68.231286	0	0.2475*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
152	XP_015775928.1	invertebrate	PREDICTED: uncharacterized protein LOC107354043 [*Acropora digitifera*]	160	0.6000	0.6500	0.7500	0.8500	The sequences were not align well				
159	XP_015759885.1	Common	PREDICTED: uncharacterized protein LOC107339154 [*Acropora digitifera*]	233	0.5352	0.4366	0.5556	0.9412	− 5567.60522	− 5618.476781	101.743122	0	1.4959*
176	XP_015752851.1	Common *up to *Homo sapiens*	PREDICTED: coiled-coil domain-containing protein 40-like [*Acropora digitifera*]	72	0.7121	0.0606	0.7143	0.0476	− 3005.915473	− 3008.484709	5.138472	0.0234	0.153*
179	XP_015779387.1	Common *up to *Homo sapiens*	PREDICTED: cilia- and flagella-associated protein 46-like [*Acropora digitifera*]	74	0.4407	0.1186	0.8500	0.0000	− 9592.908756	− 9593.981266	2.14502	0.14303	0.2771
180	XP_015779499.1	Common	PREDICTED: dynein heavy chain domain-containing protein 1-like [*Acropora digitifera*]	174	0.5634	0.6338	0.8182	0.6818	− 25,797.10645	− 25,799.75351	5.29411	0.0214	0.2641*
201	XP_015753547.1	ND	PREDICTED: coiled-coil domain-containing protein 108-like [*Acropora digitifera*]	193									

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
202	XP_015753588.1	ND	PREDICTED: acyl-CoA dehydrogenase family member 10-like [*Acropora digitifera*]	105									
203	XP_015776137.1	Common but many paralogues; Orthologues are found only in cnidarias	PREDICTED: endothelin-converting enzyme 1-like [*Acropora digitifera*]	89	0.5926	0.1111	0.7333	0.0000	− 1115.31119	− 1126.564289	22.506198	0	0.5636*
205	XP_015774822.1	Common	PREDICTED: growth arrest-specific protein 8-like [*Acropora digitifera*]	57	0.8596	0.0000	0.9375	0.0000	− 2014.578222	− 2014.646895	0.137346	0.71093	0.0338
207	XP_015765884.1	Common *up to *Homo sapiens*	PREDICTED: parkin coregulated gene protein homolog [*Acropora digitifera*]	29	0.3654	0.0192	1.0000	0.0000	− 1348.2221	− 1348.2221	0	1	0.0114
213	XP_015777297.1	Common	PREDICTED: nuclear pore complex protein Nup155-like [*Acropora digitifera*]	110	0.2857	0.0571	0.8824	0.0000	− 5947.637519	− 5947.694778	0.114518	0.73506	0.1343

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
233	XP_015775940.1	ND	PREDICTED: nuclear pore complex protein Nup153-like [*Acropora digitifera*]	116									
236	XP_015767942.1	Invertebrate	PREDICTED: trichohyalin-like [*Acropora digitifera*]	247	0.4565	0.5870	0.6667	0.7143	− 14,919.58957	− 14,936.48915	33.799176	0	0.1913*
238	XP_015777141.1	Common	PREDICTED: protein NDRG1-like [*Acropora digitifera*]	37	0.4667	0.0444	0.8947	0.0000	− 777.789269	− 778.374267	1.169996	0.2794	0.1354
259	XP_015776100.1	Common	PREDICTED: calcium uniporter protein mitochondrial-like [*Acropora digitifera*]	39	0.1111	0.0556	0.3750	0.0000	− 901.735938	− 901.861806	0.251736	0.61586	0.07
260	XP_015751166.1	ND	PREDICTED: uncharacterized protein LOC107331131 isoform X1 [*Acropora digitifera*]	160									
261	XP_015759128.1	Common	PREDICTED: malonyl-CoA decarboxylase mitochondrial-like [*Acropora digitifera*]	63	0.3934	0.0492	0.7143	0.0476	− 1258.364642	− 1268.399376	20.069468	0.00001	0.5797*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
269	XP_015779528.1	Common	PREDICTED: serine/threonine-protein phosphatase 6 regulatory subunit 3-like isoform X1 [*Acropora digitifera*]	106	0.3788	0.0455	0.8125	0.1875	− 3843.313549	− 3843.305181	0	1	0.2159
271	XP_015773788.1	Common	PREDICTED: cilia- and flagella-associated protein 46-like [*Acropora digitifera*]	58	0.4906	0.1509	0.0000	1.0000	− 12,699.84946	− 12,701.00813	2.317328	0.12794	0.2462
272	XP_015772188.1	Common	PREDICTED: dynein heavy chain 5 axonemal-like [*Acropora digitifera*]	491	0.7241	0.2874	0.9375	0.3125	− 17,517.82056	− 17,526.61787	17.594612	0.00003	0.0677*
296	XP_015759585.1	Cnidaria	PREDICTED: uncharacterized protein LOC107338846 [*Acropora digitifera*]	41	1.0000	1.0000	1.0000	1.0000	− 4122.418352	− 4126.99707 9.157436	0.00248	0.5038*	
313	XP_015764963.1	Common	PREDICTED: ras-related protein Rab-2 [*Acropora digitifera*]	24	0.9815	0.0556	1.0000	0.0000	− 1012.086212	− 1012.08622	1.60E − 05	0.99681	0
314	XP_015749660.1	Common	PREDICTED: coiled-coil domain-containing protein 40-like [*Acropora digitifera*]	49	0.7619	0.0476	0.7895	0.0526	− 3872.395629	− 3874.579337	4.367416	0.03663	0.1418*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
321	XP_015761537.1	Common	PREDICTED: apolipophorins-like [*Acropora digitifera*]	132	0.4528	0.5094	0.8824	0.8824	−6703.037392	−6710.304133	14.533482	0.00014	0.578*
322	XP_015754786.1	Common	PREDICTED: glutamate receptor 2-like [*Acropora digitifera*]	109	0.9130	0.5652	1.0000	0.0625	−5922.669025	−5937.148397	28.958744	0	0.4313*
324	XP_015778532.1	Coral	PREDICTED: uncharacterized protein LOC107356432 isoform X1 [*Acropora digitifera*]	142	0.5172	0.6552	0.8889	0.8889	−7806.236936	−7838.494356	64.51484	0	0.8648*
343	XP_015752515.1	Common	PREDICTED: uncharacterized protein LOC107332302 [*Acropora digitifera*]	123	0.4167	0.2167	0.7143	0.0476	−9341.319657	−9369.227903	55.816492	0	0.9612*
346	XP_015769652.1	Common	PREDICTED: uncharacterized protein LOC107348149 [*Acropora digitifera*]	127	0.2766	0.7660	0.3889	0.8889	−4704.683047	−4716.009591	22.653088	0	0.3596*
347	XP_015761439.1	Common	PREDICTED: tubulin beta chain-like [*Acropora digitifera*]	44	1.0000	0.0526	0.3947	0.0000	−2100.626501	−2100.62649	0	1	0.0147
354	XP_015760999.1	ND	PREDICTED: protein brambleberry-like isoform X1 [*Acropora digitifera*]	66									

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
369	XP_015778869.1	Common	PREDICTED: radial spoke head protein 4 homolog A-like [*Acropora digitifera*]	57	0.0196	0.0196	0.0000	0.0000	− 1196.417644	− 1196.417644	0	1	0.1058*
405	XP_015774007.1	Common	PREDICTED: centrosome-associated protein 350-like [*Acropora digitifera*]	316	0.6029	0.6029	0.9444	0.8333	− 19,636.16747	− 19,638.88917	5.443406	0.01964	0.3072*
406	XP_015771040.1	Common	PREDICTED: heat shock protein 75 kDa mitochondrial-like isoform X1 [*Acropora digitifera*]	83	0.0448	0.0896	0.0500	0.0000	− 2899.72292	− 2902.706387	5.966934	0.01458	0.355*
408	XP_015776834.1	ND	PREDICTED: E3 ubiquitin-protein ligase UBR4-like [*Acropora digitifera*]	326									
423	XP_015760580.1	Common	PREDICTED: uncharacterized protein LOC107339777 [*Acropora digitifera*]	113	0.3571	0.6071	0.7000	0.8000	− 6795.494372	− 6798.007913	5.027082	0.02495	0.1929*
446	XP_015763557.1	Common	PREDICTED: uncharacterized protein LOC107342572 isoform X1 [*Acropora digitifera*]	38	0.7273	0.1364	0.8933	0.1467	− 1363.705686	− 1380.40244	33.393508	0	1.7146*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
448	XP_015752532.1	ND	PREDICTED: glutamate dehydrogenase mitochondrial-like [*Acropora digitifera*]	42									
476	XP_015747763.1	Common	PREDICTED: glutamine synthetase-like [*Acropora digitifera*]	41	0.8256	0.4651	0.9375	0.0000	− 2134.15126	− 2137.67251	7.0425	0.00796	0.127*
478	XP_015774247.1	ND	PREDICTED: IQ domain-containing protein H-like isoform X1 [*Acropora digitifera*]	113									
482	XP_015779212.1	Common	PREDICTED: oral-facial-digital syndrome 1 protein-like isoform X1 [*Acropora digitifera*]	78	0.2931	0.2586	0.2941	0.0588	− 3196.620789	− 3196.621039	0.0005	0.98216	0.4421
491	XP_015752533.1	Common	PREDICTED: glutamate dehydrogenase 1 mitochondrial-like [*Acropora digitifera*]	40	0.6349	0.0794	0.7500	0.0625	− 1134.433108	− 1134.511046	0.155876	0.69298	0.0531
507	XP_015769754.1	Common	PREDICTED: alpha-aminoadipic semialdehyde dehydrogenase-like [*Acropora digitifera*]	50	0.6301	0.0000	0.7778	0.0000	− 1454.113675	− 1454.188357	0.149364	0.69914	0.1083

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
520	XP_015762433.1	ND	PREDICTED: oxysterol-binding protein 1-like isoform X1 [*Acropora digitifera*]	97									
530	XP_015754781.1	ND	PREDICTED: jouberin-like partial [*Acropora digitifera*]	116									
548	XP_015763941.1	Common	PREDICTED: mitochondrial import inner membrane translocase subunit TIM44-like [*Acropora digitifera*]	35	0.4902	0.0392	0.9375	0.0000	− 1452.847004	− 1452.885854	0.0777	0.78044	0.0708
551	XP_015751266.1	Common	PREDICTED: COP9 signalosome complex subunit 2 [*Acropora digitifera*]	52	0.7544	0.0702	0.8889	0.0000	− 2174.228102	− 2174.228172	0.00014	0.99056	0.0034
553	XP_015758581.1	Coral	PREDICTED: uncharacterized protein LOC107337859 [*Acropora digitifera*]	79	0.7826	0.7391	0.8824	0.8235	− 5942.469142	− 5944.036166	3.134048	0.07667	0.6473
557	XP_015751131.1	Common	PREDICTED: calcium uptake protein 3 mitochondrial-like [*Acropora digitifera*]	40	0.3333	0.1373	0.8125	0.0625	− 943.153162	− 943.153162	0	1	0.19

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
569	XP_015760745.1	Common	PREDICTED: WD repeat-containing protein on Y chromosome-like [*Acropora digitifera*]	106	0.4400	0.1200	0.7000	0.0000	− 1332.273696	− 1332.342163	0.136934	0.71135	0.0984
581	XP_015768680.1	Common	PREDICTED: AP-1 complex subunit beta-1-like [*Acropora digitifera*]	95	0.4286	0.0159	0.9375	0.0000	− 3600.106922	− 3600.10692	0	1	0.0433
584	XP_015754906.1	Common	PREDICTED: LOW QUALITY PROTEIN: tetratricopeptide repeat protein 41-like [*Acropora digitifera*]	134	0.8000	0.2500	0.9412	0.0588	− 4723.802784	− 4724.711713	1.817858	0.17757	0.0708
638	XP_015765830.1	Common	PREDICTED: coiled-coil domain-containing protein 180-like [*Acropora digitifera*]	39	0.4706	0.4706	0.8750	0.8125	− 4438.453722	− 4439.852736	2.798028	0.09438	0.1469
689	XP_015779505.1	Common	PREDICTED: T-complex protein 1 subunit epsilon-like [*Acropora digitifera*]	50	0.4151	0.0000	1.0000	0.0000	− 1349.069975	− 1349.069975	0	1	0.0126
700	XP_015771206.1	Common	PREDICTED: LOW QUALITY PROTEIN: dynein heavy chain 5 axonemal-like [*Acropora digitifera*]	456	0.8491	0.2453	0.8333	0.0000	− 21,901.37742	− 21,901.41719	0.079528	0.77794	0.0434

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
701	XP_015764594.1	Common	PREDICTED: multidrug resistance-associated protein 1-like partial [*Acropora digitifera*]	138	0.0667	0.2500	0.0000	0.2889	− 5314.559958	− 5316.488854	3.857792	0.04952	0.0756*
702	XP_015749541.1	Common	PREDICTED: dynein heavy chain 5 axonemal-like [*Acropora digitifera*]	241	0.7333	0.3091	0.8491	0.2264	− 19,914.04464	− 19,914.03897	0	1	0.0436
704	XP_015761526.1	Common	PREDICTED: uncharacterized protein LOC107340675 [*Acropora digitifera*]	384	0.5273	0.6727	0.6364	0.6818	− 14,494.9916	− 14,502.81881	15.654412	0.00008	0.558*
707	XP_015776648.1	coral	PREDICTED: uncharacterized protein LOC107354682 [*Acropora digitifera*]	53	0.7000	1.0000	0.8235	1.0000	− 2206.6611	− 2211.4453	9.5684	0.00198	0.5574*
708	XP_015769058.1	invertebrate	PREDICTED: uncharacterized protein LOC107347608 isoform X1 [*Acropora digitifera*]	23	0.6154	0.0000	0.8125	0.0000	− 411.330933	− 412.569958	2.47805	0.11545	0.7416
715	XP_015758851.1	mostly Cnidaria	PREDICTED: uncharacterized protein LOC107338130 [*Acropora digitifera*]	34	0.5250	0.8000	0.8182	0.8636	− 1039.534647	− 1047.217981	15.366668	0.00009	0.5813*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
737	XP_015775871.1	invertebrate	PREDICTED: uncharacterized protein LOC107353986 [*Acropora digitifera*]	19	0.2963	0.0185	0.4138	0.0000	− 501.931143	− 501.931143	0	1	0.2258
794	XP_015762546.1	Common	PREDICTED: fas-binding factor 1-like [*Acropora digitifera*]	96	0.6327	0.1837	0.8333	0.0000	− 8440.113709	− 8441.896721	3.566024	0.05897	0.4328
820	XP_015758136.1	Common	PREDICTED: serine-rich adhesin for platelets-like isoform X1 [*Acropora digitifera*]	245	0.6327	0.1837	0.8333	0.0000	− 12,802.35912	− 12,812.27858	19.838918	0.00001	0.6359*
843	XP_015757599.1	Common	PREDICTED: uncharacterized protein LOC107337006 [*Acropora digitifera*]	78	0.3673	0.5306	0.5200	0.5600	− 2872.720761	− 2881.604256	17.76699	0.00002	0.6082*
855	XP_015770511.1	ND	PREDICTED: uncharacterized protein LOC107348935 isoform X1 [*Acropora digitifera*]	180									
860	XP_015774349.1	Common	PREDICTED: uncharacterized protein LOC107352540 [*Acropora digitifera*]	111	0.4098	0.2459	0.7000	0.0500	− 3435.261669	− 3435.269125	0.014912	0.90281	0.1495

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
877	XP_015755286.1	Common	PREDICTED: nuclear pore complex protein Nup214-like [*Acropora digitifera*]	60	0.5500	0.0250	0.7273	0.0000	− 4018.397794	− 4025.978777	15.161966	0.0001	0.7878*
981	XP_015752400.1	Common	PREDICTED: E3 ubiquitin-protein ligase HECTD1-like partial [*Acropora digitifera*]	318	0.5738	0.1803	0.7143	0.0000	− 13,250.51217	− 13,250.51234	0.000332	0.98546	0.0492
983	XP_015758664.1	Mostly coral	PREDICTED: uncharacterized protein LOC107337932 [*Acropora digitifera*]	29	0.9130	1.0000	0.8235	0.9412	The sequences were not align well				
986	XP_015747953.1	Common	PREDICTED: phosphofurin acidic cluster sorting protein 2-like [*Acropora digitifera*]	89	0.3175	0.5873	0.7778	0.7778	− 3557.745589	− 3557.745589	0	1	0.0797
989	XP_015766222.1	Common	PREDICTED: intracellular protein transport protein USO1-like [*Acropora digitifera*]	69	0.3333	0.3750	0.5000	0.5000	− 3358.019255	− 3358.63916	1.23981	0.26551	0.1494
991	XP_015756131.1	Common	PREDICTED: CTP synthase 1-like [*Acropora digitifera*]	59	0.4167	0.0278	0.8889	0.0000	− 3279.169078	− 3279.918259	1.498362	0.22092	0.1149

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
996	XP_015752902.1	Common	PREDICTED: V-type proton ATPase 116 kDa subunit a isoform 1-like [*Acropora digitifera*]	104	0.4811	0.0094	0.7273	0.0000	− 2721.364847	− 2727.665941	12.602188	0.00039	0.3906*
1028	XP_015773662.1	Common	PREDICTED: proteasome subunit alpha type-2 [*Acropora digitifera*]	26	0.5306	0.1633	0.8750	0.0000	− 1236.421722	− 1236.430977	0.01851	0.89178	0.0203
1047	XP_015767289.1	Common	PREDICTED: LOW QUALITY PROTEIN: patatin-like phospholipase domain-containing protein 7 [*Acropora digitifera*]	155	0.4483	0.5517	0.7368	0.7895	− 7128.555716	− 7128.611161	0.11089	0.73913	0.1
1049	XP_015776130.1	Common	PREDICTED: uncharacterized protein LOC107354198 [*Acropora digitifera*]	29	0.3881	0.0896	0.4667	0.0000	− 2620.371052	− 2620.371052	0	1	0.1518
1081	XP_015752463.1	Common	PREDICTED: alcohol dehydrogenase [NADP(+)]-like isoform X1 [*Acropora digitifera*]	36	0.0526	0.0105	0.0000	0.0000	− 2107.802857	− 2109.228794	2.851874	0.09127	0.2116
1112	XP_015751662.1	Common	PREDICTED: uncharacterized protein LOC107331568 [*Acropora digitifera*]	93	0.6087	0.7609	0.8333	0.8889	− 3390.944981	− 3392.210567	2.531172	0.11162	0.4079

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1148	XP_015773720.1	Common	PREDICTED: coiled-coil domain-containing protein 169-like [*Acropora digitifera*]	31	0.5067	0.6733	0.6393	0.7705	−987.817708	−987.877536	0.119656	0.72941	0.1573
1169	XP_015751110.1	Common	PREDICTED: neutralized-like protein 4 [*Acropora digitifera*]	25	0.3571	0.0714	0.6250	0.0000	−969.879431	−969.879431	0	1	0.0421
1188	XP_015750023.1	Common	PREDICTED: tetratricopeptide repeat protein 7B-like [*Acropora digitifera*]	93	0.2396	0.1458	0.5000	0.2188	−467.662445	−467.662445	0	1	0.1941
1192	XP_015777990.1	Common	PREDICTED: phosphatidylinositide phosphatase SAC2-like [*Acropora digitifera*]	134	0.5273	0.3455	0.7778	0.5556	−6633.793781	−6634.254901	0.92224	0.33689	0.2395
1208	XP_015779881.1	Common	PREDICTED: adenylate kinase 2 mitochondrial-like [*Acropora digitifera*]	28	0.4912	0.0526	0.5455	0.0455	−917.585296	−917.585296	0	1	0.0874
1219	XP_015750990.1	Common	PREDICTED: sorcin-like [*Acropora digitifera*]	14	0.3750	0.1042	0.7500	0.0000	−973.351282	−973.351282	0	1	0.1201
1240	XP_015762409.1	Common	PREDICTED: uncharacterized protein LOC107341498 [*Acropora digitifera*]	43	0.9512	0.0244	0.8000	0.0000	−979.540977	−979.540977	0	1	0.0043

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1244	XP_015778046.1	Common	PREDICTED: IQ domain-containing protein K-like [*Acropora digitifera*]	31	0.5472	0.0566	0.9375	0.0000	− 705.242158	− 705.244806	0.005296	0.94199	0.1654
1265	XP_015756087.1	Common	PREDICTED: LOW QUALITY PROTEIN: ATP-binding cassette sub-family B member 7 mitochondrial-like [*Acropora digitifera*]	60	0.3500	0.0500	0.8750	0.0000	− 3456.964265	− 3456.978724	0.028918	0.86497	0.0562
1304	XP_015771068.1	Common	PREDICTED: leucine-rich repeat-containing protein 34-like [*Acropora digitifera*]	25	0.3396	0.0566	0.5294	0.0000	− 1202.292257	− 1202.82842	1.072326	0.30042	0.3425
1313	XP_015767620.1	Mostly coral	PREDICTED: uncharacterized protein LOC107346341 [*Acropora digitifera*]	21	0.5455	0.0909	0.8333	0.0000	− 891.6031	− 891.93378	0.66136	0.41608	0.5578
1383	XP_015778298.1	Common	PREDICTED: centrosomal protein of 78 kDa-like [*Acropora digitifera*]	36	0.6552	0.4310	0.9333	0.9333	− 3216.152152	− 3216.451526	0.598748	0.43906	0.1488
1384	XP_015778638.1	Common	PREDICTED: disintegrin and metalloproteinase domain-containing protein 10-like [*Acropora digitifera*]	69	0.4265	0.4853	0.8333	0.7778	− 4452.680312	− 4477.27061	49.180596	0	0.3919*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1425	XP_015766566.1	Common	PREDICTED: dipeptidyl peptidase 9-like [*Acropora digitifera*]	79	0.5357	0.0357	0.8235	0.0000	− 1294.103975	− 1294.829025	1.4501	0.22851	0.0268
1429	XP_015771548.1	Common	PREDICTED: uncharacterized protein LOC107349871 [*Acropora digitifera*]	195	0.6047	0.8140	0.8333	0.8333	− 8887.03239	− 8887.526485	0.98819	0.32019	0.5109
1441	XP_015769682.1	Common	PREDICTED: maestro heat-like repeat-containing protein family member 1 isoform X1 [*Acropora digitifera*]	165	0.2593	0.0988	0.6000	0.0000	− 7756.809569	− 7756.809569	0	1	0.1836
1453	XP_015757037.1	Common	PREDICTED: sodium bicarbonate transporter-like protein 11 [*Acropora digitifera*]	78	0.3889	0.1667	0.8824	0.0588	− 5053.393986	− 5056.786249	6.784526	0.0092	0.143*
1483	XP_015768017.1	Common	PREDICTED: ubiquitin-conjugating enzyme E2 N-like [*Acropora digitifera*]	17	0.3455	0.0364	0.9286	0.0000	− 866.379895	− 866.379895	0	1	0.0173
1488	XP_015756542.1	Common	PREDICTED: coenzyme Q-binding protein COQ10 homolog B mitochondrial-like isoform X1 [*Acropora digitifera*]	31	0.3077	0.0962	0.9286	0.0000	− 1218.345451	− 1218.443154	0.195406	0.65845	0.1062

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1537	XP_015763600.1	Common	PREDICTED: ral GTPase-activating protein subunit beta-like partial [*Acropora digitifera*]	130	0.3243	0.5541	0.8824	0.8824	− 8206.246335	− 8206.307537	0.122404	0.72644	0.1515
1558	XP_015765929.1	Common	PREDICTED: reticulon-4-interacting protein 1 mitochondrial-like isoform X1 [*Acropora digitifera*]	29	0.3273	0.0909	0.9333	0.0000	− 1238.511769	− 1239.457472	1.891406	0.16904	0.138
1583	XP_015775101.1	Common	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3-like isoform X1 [*Acropora digitifera*]	20	0.1163	0.0233	0.1250	0.0000	− 345.704366	− 348.596999	5.785266	0.01616	0.4373*
1586	XP_015752475.1	mostly Cnidaria	PREDICTED: uncharacterized protein LOC107332261 [*Acropora digitifera*]	26	0.0345	0.0000	0.0000	0.0000	− 1351.62488	− 1351.624895	3.00E − 05	0.99563	0.2517
1657	XP_015759760.1	Common	PREDICTED: uncharacterized HIT-like protein slr1234 [*Acropora digitifera*]	19	0.4390	0.0000	0.9375	0.0000	− 912.379219	− 912.379219	0	1	0.0812
1684	XP_015752772.1	Common	PREDICTED: uncharacterized protein LOC107332547 partial [*Acropora digitifera*]	74	0.2381	0.1905	0.3684	0.0526	− 4036.599743	− 4047.314959	21.430432	0	0.4134*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1687	XP_015752764.1	Common	PREDICTED: uncharacterized protein LOC107332535 [*Acropora digitifera*]	44	0.6061	0.6667	1.0000	0.9375	− 8757.864703	− 8758.396364	1.063322	0.30246	0.3202
1715	XP_015752732.1	ND	PREDICTED: centrosomal protein of 164 kDa-like [*Acropora digitifera*]	33									
1716	XP_015748698.1	Common	PREDICTED: uncharacterized methyltransferase Mb3374-like [*Acropora digitifera*]	31	0.5000	0.0294	0.9375	0.0000	− 1545.002639	− 1545.339153	0.673028	0.412	0.2776
1720	XP_015747295.1	Common	PREDICTED: cytosolic acyl coenzyme A thioester hydrolase-like [*Acropora digitifera*]	40	0.6744	0.1163	1.0000	0.0000	− 1750.769164	− 1750.776092	0.013856	0.9063	0.1091
1721	XP_015764849.1	Common	PREDICTED: mitochondrial carnitine/ acylcarnitine carrier-like protein [*Acropora digitifera*]	31	0.7222	0.0278	0.6667	0.0000	− 436.111597	− 436.111597	0	1	0.216
1741	XP_015763949.1	Common	PREDICTED: RNA-binding protein 8A-like [*Acropora digitifera*]	19	0.2045	0.1364	1.0000	0.0000	− 727.336659	− 727.336659	0	1	0.0175
1742	XP_015769432.1	Common	PREDICTED: intersectin-1-like [*Acropora digitifera*]	149	0.1778	0.0444	0.4211	0.0000	− 1584.113433	− 1584.113433	0	1	0.127

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1791	XP_015761375.1	Common	PREDICTED: uncharacterized protein C17orf98-like partial [*Acropora digitifera*]	9	0.9070	0.0698	0.9333	0.0000	− 1164.728103	− 1164.728103	0	1	0.4016
1828	XP_015751652.1	Common	PREDICTED: testis prostate and placenta-expressed protein-like [*Acropora digitifera*]	32	0.1316	0.0000	0.0667	0.0000	− 968.414655	− 968.413423	0	1	0.1202
1867	XP_015756062.1	Common	PREDICTED: uncharacterized protein LOC107335558 [*Acropora digitifera*]	51	0.8148	0.1852	0.9444	0.0000	− 2587.669413	− 2593.038437	10.738048	0.00105	0.5256*
1889	XP_015779568.1	ND	PREDICTED: coadhesin-like [*Acropora digitifera*]	39									
1894	XP_015757089.1	Common	PREDICTED: uncharacterized protein LOC107336526 [*Acropora digitifera*]	42	0.4255	0.0851	0.8824	0.0000	− 4685.127541	− 4691.377889	12.500696	0.00041	0.2221*
1909	XP_015750069.1	Common	PREDICTED: trichohyalin-like [*Acropora digitifera*]	44	0.6471	0.8235	0.9375	1.0000	− 8074.487447	− 8083.289363	17.603832	0.00003	0.3479*
1914	XP_015750707.1	Common	PREDICTED: tumor susceptibility gene 101 protein-like [*Acropora digitifera*]	38	0.2386	0.0568	0.5000	0.0000	− 2870.079767	− 2880.617227	21.07492	0	0.5166*

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora*_RRXT	*Acropora*_RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
1942	XP_015761955.1	Common	PREDICTED: putative uncharacterized protein CXorf58 [*Acropora digitifera*]	26	0.6087	0.0435	1.0000	0.0000	− 1598.860969	− 1598.862705	0.003472	0.95301	0.3219
1945	XP_015764932.1	Common	PREDICTED: serine/arginine repetitive matrix protein 2-like [*Acropora digitifera*]	139	0.0233	0.0465	0.0333	0.0333	− 2733.001077	− 2733.00955	0.016946	0.89643	0.2079
1954	XP_015769753.1	Common	PREDICTED: alpha-aminoadipic semialdehyde dehydrogenase-like [*Acropora digitifera*]	21	0.8197	0.0000	1.0000	0.0000	− 2452.086114	− 2452.168903	0.165578	0.68407	0.0755
1965	XP_015779912.1	ND	PREDICTED: coiled-coil domain-containing protein 78-like partial [*Acropora digitifera*]	86									
1993	XP_015775713.1	Common	PREDICTED: serine/arginine-rich splicing factor 10-like isoform X1 [*Acropora digitifera*]	26	0.4314	0.8039	1.0000	1.0000	− 1231.638813	− 1231.638811	0	1	0.0253
2001	XP_015764471.1	Common	PREDICTED: tubby protein homolog partial [*Acropora digitifera*]	24	0.4091	0.1061	1.0000	0.0000	− 2267.547227	− 2268.358159	1.621864	0.20283	0.0984

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
2011	XP_015757240.1	Common	PREDICTED: coiled-coil domain-containing protein 34-like [*Acropora digitifera*]	41	0.4000	0.1250	1.0000	0.0000	− 2217.367086	− 2218.696315	2.658458	0.103	0.2999
2033	XP_015760757.1	Common	PREDICTED: protein furry homolog-like [*Acropora digitifera*]	359	0.4651	0.5814	0.7273	0.6818	− 14,592.19179	− 14,593.78312	3.182664	0.07442	0.1183
2077	XP_015770207.1	Common	PREDICTED: uncharacterized protein LOC107348672 [*Acropora digitifera*]	69	0.0392	0.0000	0.0294	0.0000	− 2073.477159	− 2075.44869	3.943062	0.04707	0.6536*
2102	XP_015759488.1	Common	PREDICTED: LOW QUALITY PROTEIN: ubiquitin thioesterase Zranb1-like [*Acropora digitifera*]	79	0.2778	0.1852	0.8333	0.0000	− 3688.252784	− 3688.399076	0.292584	0.58857	0.0841
2141	XP_015758774.1	Common	PREDICTED: putative nuclease HARBI1 [*Acropora digitifera*]	37	0.4000	0.0500	0.4783	0.0000	The sequences were not align well				
2143	XP_015747929.1	Common	PREDICTED: chromodomain Y-like protein 2 [*Acropora digitifera*]	53	0.8750	0.7500	0.8235	0.8235	− 2765.413717	− 2766.951565	3.075696	0.07947	0.2305
2186	XP_015759732.1	Common	PREDICTED: ferrochelatase mitochondrial-like [*Acropora digitifera*]	31	0.3519	0.0556	0.6111	0.0000	− 2028.052468	− 2028.052883	0.00083	0.97702	0.0936

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
2197	XP_015763809.1	Common	PREDICTED: centrosomal protein of 41 kDa-like [*Acropora digitifera*]	25	0.2549	0.0980	0.6875	0.1250	− 2227.010882	− 2229.658084	5.294404	0.02139	0.1863*
2229	XP_015750476.1	Common	PREDICTED: uncharacterized protein KIAA1109-like partial [*Acropora digitifera*]	20	0.7556	0.9556	1.0000	1.0000	− 24,470.74651	− 24,470.74652	6.00E-06	0.99805	0.165
2292	XP_015770625.1	Common	PREDICTED: protein FAM91A1-like [*Acropora digitifera*]	68	0.6667	0.0000	0.9444	0.0000	− 4618.531127	− 4618.59409	0.125926	0.72269	0.1124
2335	XP_015765239.1	Mostly Cnidaria	PREDICTED: uncharacterized protein LOC107343818 [*Acropora digitifera*]	80	0.6154	0.1026	0.9412	0.0588	− 4039.04211	− 4039.959758	1.835296	0.1755	0.2935
2343	XP_015772286.1	ND	PREDICTED: uncharacterized protein LOC107350564 [*Acropora digitifera*]	40									
2361	XP_015747921.1	Common	PREDICTED: GMP reductase 1-like [*Acropora digitifera*]	35	0.7455	0.0364	1.0000	0.0000	− 1747.132047	− 1747.132047	0	1	0.0439
2366	XP_015769306.1	Common	PREDICTED: uncharacterized protein LOC107347846 [*Acropora digitifera*]	199	0.5000	0.7500	0.5758	0.7879	− 10,873.17513	− 10,902.25267	58.155094	0	0.5182

Table 1 (continued)

Protein No	Accession number	Conservation among the animals	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_ RRXT	All_ RRXS	*Acropora* _RRXT	*Acropora* _RRXS	Model8	Model8a	Δln*L*	*P*	d*N*/d*S*
2393	XP_015761217.1	Common	PREDICTED: DENN domain-containing protein 5A-like [*Acropora digitifera*]	100	0.3214	0.1429	0.1765	0.0000	− 6883.329708	− 6884.102644	1.545872	0.21375	0.0909
2397	XP_015758140.1	Mostly Cnidaria	PREDICTED: uncharacterized protein LOC107337485 [*Acropora digitifera*]	40	0.5161	0.2258	0.8750	0.0000	− 2088.218097	− 2090.538197	4.6402	0.03123	0.353*
2410	XP_015752728.1	Common	PREDICTED: kinesin-like protein KIF3B isoform X1 [*Acropora digitifera*]	40	0.6000	0.5667	0.7500	0.6250	− 4895.055861	− 4895.18087	0.250018	0.61706	0.1534
2419	XP_015754940.1	Common	PREDICTED: protein FAM91A1-like [*Acropora digitifera*]	70	0.3939	0.0152	0.8095	0.0000	− 4550.529523	− 4550.903965	0.748884	0.38683	0.1076
2434	XP_015777195.1	Common	PREDICTED: exocyst complex component 4-like [*Acropora digitifera*]	28	0.3929	0.1250	0.6522	0.0000	− 2268.204693	− 2268.202989	0	1	0.1435

Table 2 Isolated PKA substrates in sperm proteins matched with PhosphoSitePlus Substrates of Kinases

Protein No	Accession number	Conservation	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora* _RRXT	*Acropora* _RR	Model 8	Model 8a	Δln*L*	*P*	d*N*/d*S*
238	XP_015777141.1	Common	PREDICTED: protein NDRG1-like [*Acropora digitifera*]	37	0.4667	0.0444	0.8947	0.0000	− 777.789269	− 778.374267	1.169996	0.2794	0.1354
406	XP_015771040.1	Common	PREDICTED: heat shock protein 75 kDa mitochondrial-like isoform X1 [*Acropora digitifera*]	83	0.0448	0.0896	0.0500	0.0000	− 2899.72292	− 2902.706387	5.966934	0.01458	0.355*
491	XP_015752533.1	Common	PREDICTED: glutamate dehydrogenase 1 mitochondrial-like [*Acropora digitifera*]	40	0.6349	0.0794	0.7500	0.0625	− 1134.433108	− 1134.511046	0.155876	0.69298	0.0531
689	XP_015779505.1	Common	PREDICTED: T-complex protein 1 subunit epsilon-like [*Acropora digitifera*]	50	0.4151	0.0000	1.0000	0.0000	− 1349.069975	− 1349.069975	0	1	0.0126

Table 2 (continued)

Protein No	Accession number	Conservation	Name	M_W (kDa)	Conservation rates of PKA phosphorylation residues (RR*T/S) CodeML Analyses								
					All_RRXT	All_RRXS	*Acropora* _RRXT	*Acropora* _RR	Model 8	Model 8a	Δln*L*	*P*	d*N*/d*S*
991	XP_015756131.1	Common	PREDICTED: CTP synthase 1-like [*Acropora digitifera*]	59	0.4167	0.0278	0.8889	0.0000	− 3279.169078	− 3279.918259	1.498362	0.22092	0.1149
1219	XP_015750990.1	Common	PREDICTED: sorcin-like [*Acropora digitifera*]	14	0.3750	0.1042	0.7500	0.0000	− 973.351282	− 973.351282	0	1	0.1201
1993	XP_015775713.1	Common	PREDICTED: serine/arginine-rich splicing factor 10-like isoform X1 [*Acropora digitifera*]	26	0.4314	0.8039	1.0000	1.0000	− 1231.638813	− 1231.638811	0	1	0.0253

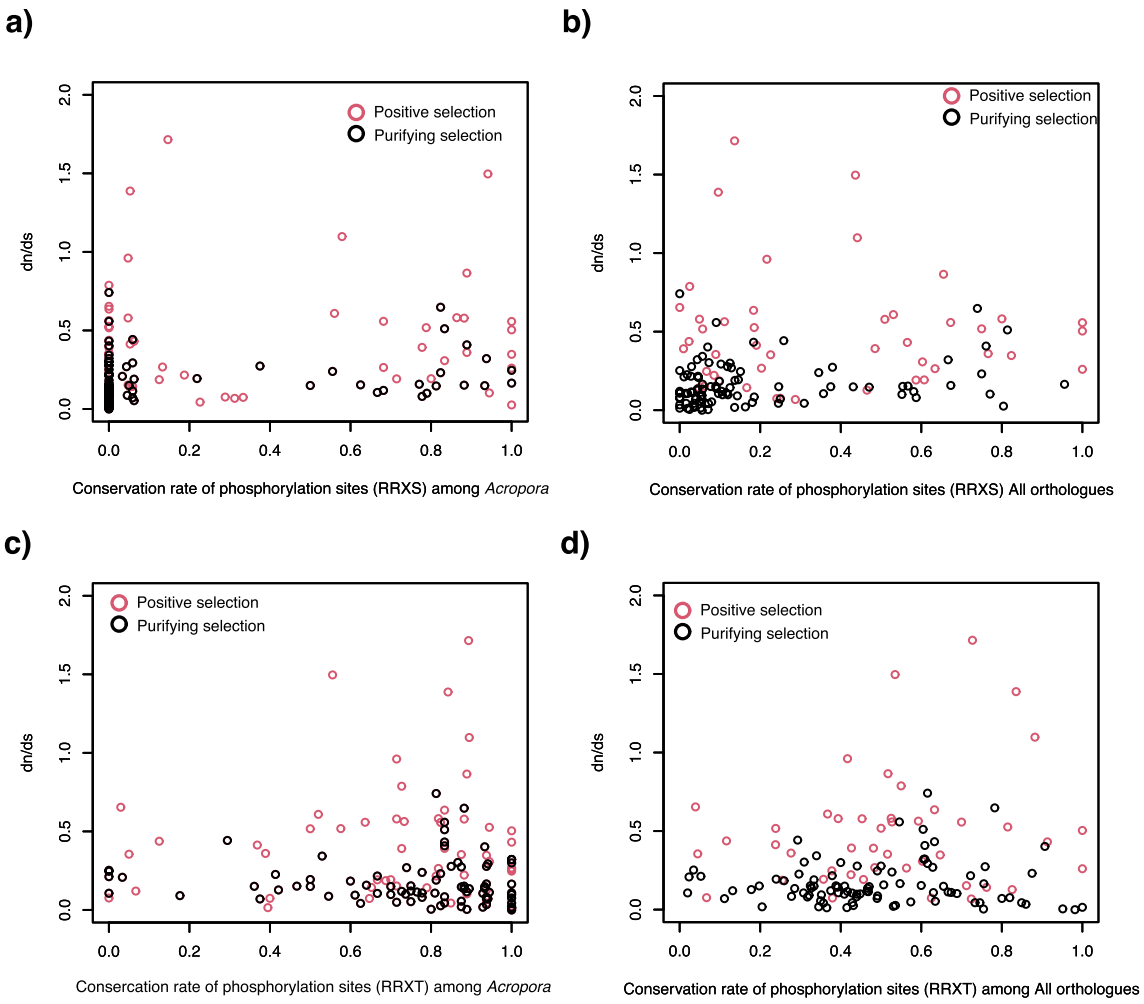

Fig. 3 Conservation rates of serine or threonine residues (RRXS/T) and d*N*/d*S* ratio among *Acropora* or all isolated orthologs. The relationship between serine or threonine residue conservation rates and the d*N*/d*S* ratio output in the CodeML analyses in model8 (positive selection) was utilized for plotting. Conservation rates of **a** RRXS in all orthologs, **b** *Acropora*, or **d** RRXT in all orthologs, and **c** *Acropora* were plotted

animals. Notably, proteins essential for flagellar motility, such as dyneins, are also under positive selection. PKA is conserved across animals, and many candidate substrates exhibit conservation between mammals and phylogenetically early branching animal cnidarians, including corals (Barott et al. 2013; Glass et al. 2023; Speer et al. 2021). Proteins essential for the architecture of motility apparatus axonemes, such as dyneins and β-tubulin, feature PKA phosphorylation sequences. These findings align with those of prior studies (Glass et al. 2023; Speer et al. 2021), and we identified downstream candidate PKA substrates. While PKA and adenylyl cyclase are conserved, signal transduction pathways have evolved through speciation (Bradley and Beltrao 2019). For example, in mammals, mitochondrial PKA controls sperm motility via ATP (Mizrahi and Breitbart 2014). In contrast, valinomycin experiments indicate membrane hyperpolarization commonly contributes to activating soluble adenylyl cyclase (sAC) in the coral *Acropora* spp. and ascidians (Izumi et al. 1999). Although several mitochondrial proteins, including adenylate kinase 2 and other candidate PKA substrates, were identified in the coral *A. digitifera*, we did not observe PKA phosphorylation in glucose-6-phosphate isomerase, which is involved in ATP synthesis in mammals (Mizrahi and Breitbart 2014). Although we identified the sperm proteins from only one colony of *A. digitifera* and the amount of the sperm proteins potentially changes according to the genotype of the colonies, this is the first step to understanding the sperm flagellar motility activation in the coral *Acropora* spp.

Although the overlap between the substrates and the database was low, we identified the dynein light chain, essential for the flagellar motility initiation among vertebrates. Additionally, the flagellar-specific proteins, such as radial spoke head 4 proteins (RSP4), were not included

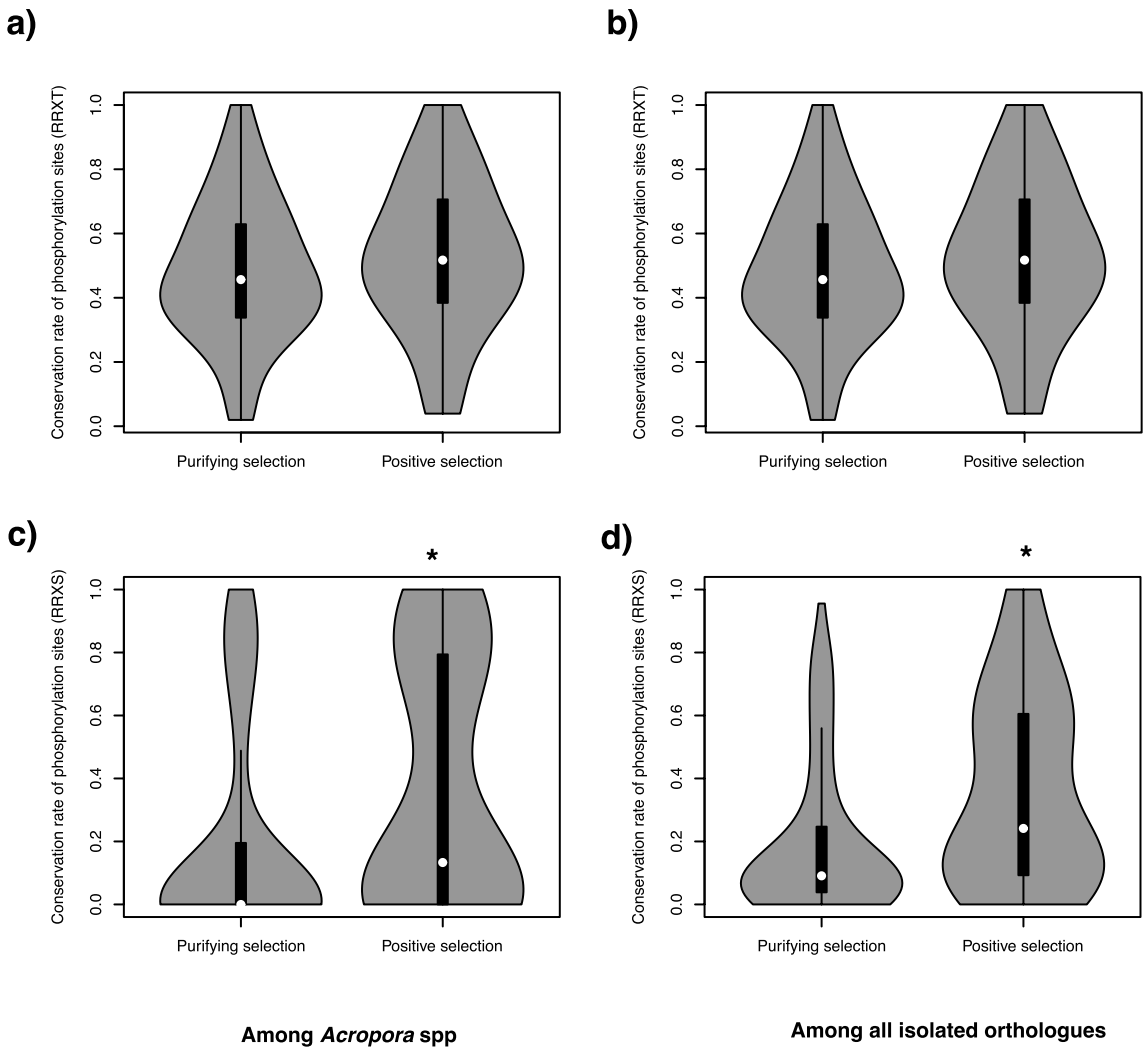

Fig. 4 Conservation rates of serine or threonine residues (RRXS/T) between under positive selection and purifying selection. Violin plots illustrating RRXT or TTXS conservation rates between orthologs under positive and purifying selection conditions. **a** RRXT among *Acropora*, **b** all orthologs, **c** RRXS among *Acropora*, and **d** all orthologs. Differences were compared using the Wilcoxon rank-sum test with continuity correction ($W = 1425$, $P < 0.0005$ in RRXS *Acropora*, and $W = 1361.5$, $P < 0.0005$ in RRTS in all orthologs). *Significant difference between positive and purifying selections (Wilcoxon rank-sum test)

in the database; however, RSP3, a paralog of RSP4, was downstream of PKA phosphorylation signaling and regulates dynein activity (Bicka et al. 2023; Gaillard et al. 2001). There are many flagellar-specific proteins involved in flagellar motility activation, with many uncharacterized proteins presumed to be involved as well. Nevertheless, identifying these proteins involved in flagellar motility initiation, using techniques such as an anti-phospho PKA substrate antibody, is necessary.

Ubiquitin ligases are present in sperm proteins and are mostly involved in flagellar formation during spermatogenesis (Long et al. 2015). Ubiquitination during cilia and flagella formation, as summarized previously (Long et al. 2015), involves ubiquitination of motor proteins during cilia and flagella formation and disassembly (Huang et al. 2009). The activation of flagellar motility could be influenced by ubiquitination, as proteasomes cause proteolysis of ubiquitinated proteins. In salmonid fish sperm flagella, proteasomes form a complex with the motor protein dynein, as predicted by PKA phosphorylation (Inaba et al. 1998). PKA and proteasomes are coupled to function together for capacitation in mammalian sperm via phosphorylation (Zapata-Carmona et al. 2019). Moreover, proteasomes are present in the sperm of the coral *A. digitifera*. Thus, the PKA phosphorylation-induced, proteasome-dependent motility regulatory mechanism potentially contributes to the activation of flagellar motility.

In the present study, our findings revealed that one-third of the PKA substrate candidates underwent positive selection, indicating adaptive evolution and functional

modification. Orthologs of many PKA substrate candidates in *Acropora* have been conserved through speciation and evolution in the animal kingdom. Positive selection is favored when functional modifications benefit sperm function. For example, faster-swimming sperm may have a competitive advantage in synchronous spawning (Fitzpatrick 2020; Locatello et al. 2007). Additionally, sneaking males can release sperm to potentially enhance fertilization success (Birkhead and Pizzari 2002; Burness et al. 2004). In other words, proteins associated with sperm function, including flagellar motility, likely underwent adaptive evolution. Despite the validity of these speculations, the identification and characterization of the phosphorylation status of flagellar proteins remain crucial.

In the coral *Acropora*, PKA substrates play a role in sperm motility activation and chemotaxis. *Acropora* is a multispecies spawning cnidarian (Baird et al. 2021b; Hayashibara et al. 1993), one of a species-abundant genus of over 110 species identified in the Indo-Pacific (Wallace 1999), and many sympatric species spawn synchronously (Baird et al. 2021b). The initiation of sperm flagellar motility after spawning occurs after the segregation of gamete bundles into sperm and eggs (Morita et al. 2006). The timing of sperm flagellar motility initiation and regulation of flagellar beating, such as chemotaxis, may facilitate contact with conspecifics in a mixture of heterospecifics. The multispecies spawning patterns may have influenced the evolution of flagellar motility, enhancing fertilization success and fitness. Additionally, PKA signaling pathways are conserved among cnidarians, and the conservation of phosphorylation residues in orthologs under positive selection is higher than that under purifying selection. These features are presumably associated with PKA-dependent sperm function to adapt to reproduction.

The higher conservation rates of the serine residues may be attributed to the strict positioning of the threonine residues in terms of the substrate (Pandey et al. 2023) and the structure of the kinase (Durek et al. 2009). PKA substrates under positive selection presumably underwent changes in the 3D structure of the proteins owing to amino acid substitutions. In the present study, the conservation rates of the threonine residues were not significantly different between positive selection and purifying selection. This could be because the threonine residues are structurally highly ordered for phosphorylation. Thus, conformational changes of the proteins induced by positive selection presumably influence phosphorylation by PKA. Additionally, the phosphorylation preferences of serine residues by PKA are reported higher than those of the threonine owing to their phosphoacceptor preferences (Chen et al. 2014). The number of threonine residues available for phosphorylation has been reported to be considerably lower, approximately one-third, than that of serine residues (Kreegipuu et al. 1998), which is consistent with our analyses.

In the present study, seven candidates of PKA substrates that matched with the PhosphoSitePlus Substrate of Kinase database were under purifying selection. Functional modifications are presumably not favored in common substrates that function across various tissues. Nevertheless, distinctive phosphorylation sites contribute to selective phosphorylation in the progression of the cell cycle, an essential process in all types of cells (Alexander et al. 2011). Although PKA signal transduction functions from prokaryotes to eukaryotes (Portela and Rossi 2020), and phosphorylation motifs are conserved among eukaryotes (Bradley and Beltrao 2019), the substrates in the flagellar motility did not overlap with the PhosphoSitePlus Substrate of Kinase database. Moreover, the PKA substrate for motility activation is specific to physiological reactions; thus, the substrates are potentially different. However, further investigations are required to confirm this.

Cnidarian- or coral-specific proteins are likely specialized in corals. Most of these cnidarian or coral-specific proteins were uncharacterized proteins. Therefore, identifying the functions of these proteins and their localization within coral polyps is imperative. As previously mentioned, the predictability of multispecies spawning and flagellar motility contributing to fertilization success with conspecifics has yet to be examined in terms of the functions of these proteins.

The applied membrane-permeable cAMP facilitated motility initiation, highlighting the need for further detailed analyses to confirm the isolated PKA substrate in this study. Although only in silico analyses were used to identify the PKA substrate, immunoprecipitation and examination of the phosphoprotein state before and after motility activation with an anti-PKA phosphorylation antibody are crucial. Additionally, new techniques are available for identifying novel PKA substrates; the PKA and substrate complex can be analyzed using proximity-dependent biotin identification (BioID) methods (Niinae et al. 2021). Preliminary experiments using membrane-permeable cAMP analogs demonstrated flagellar motility activation (Fig. 2), consistent with previous detailed biochemical studies in corals (Glass et al. 2023; Speer et al. 2021). While inhibitor analyses will be convenient for identifying axonemal protein phosphorylation pathways via immunoblot after 2D electrophoresis, the separation of membrane proteins with 2D electrophoresis may pose challenges. As discussed earlier, only a limited number of candidates overlapped with the PKA substrate registered in the PhosphoSitePlus Substrate of Kinase database. Thus, comprehensive analyses are essential to provide more reliable information.

In conclusion, we used in silico analyses to isolate PKA substrate candidates, exploring how orthologs of the candidate proteins are conserved and how orthologs are under

positive selection, leading to adaptive evolution. However, molecular evolutionary analyses, such as hypotheses related to internal fertilization-specific positive selection or high intensity of sperm competition via branch residue analyses, could not be conducted owing to sequence diversity in aligning nucleotide files. Nevertheless, the higher conservation of phosphorylation residues in orthologs under positive selection suggests the evolution of specific regions in the PKA substrate protein to acquire desirable functions, accompanied by the conservation of serine and threonine residues for PKA phosphorylation. Furthermore, this study implies the potential conservation of PKA signal transduction, with its functions evolving under various selective pressures, such as sperm competition or environmental changes from external to internal fertilization. This study provides insights into the evolutionary dynamics of reproductive mechanisms in corals, the potential adaptive evolution of sperm-related proteins, and the conservation of PKA signal transduction across diverse reproductive conditions. Additionally, the study may contribute to a better understanding of the molecular mechanisms underlying fertilization success, which could have implications for coral reproductive ecology, evolution, and conservation. However, we did not investigate these mechanisms in flagellar proteins or examine their phosphorylation during motility activation. Therefore, future efforts should focus on identifying flagellar proteins among the candidates and elucidating their phosphorylation during flagellar motility activation in spawning coral *Acropora*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00239-024-10168-x.

Funding This work was supported by a JSPS KAKENHI Grant (17K07414, 21H05304 to MM).

Declarations

Conflict of interest The authors have no conflicts of interest.

References

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci USA 107:20400

Alexander J, Lim D, Joughin BA, Hegemann B, Hutchins JR, Ehrenberger T, Ivins F, Sessa F, Hudecz O, Nigg EA, Fry AM, Musacchio A, Stukenberg PT, Mechtler K, Peters JM, Smerdon SJ, Yaffe MB (2011) Spatial exclusivity combined with positive and negative selection of phosphorylation motifs is the basis for context-dependent mitotic signaling. Sci Signal 4:ra42

Alvau A, Battistone MA, Gervasi MG, Navarrete FA, Xu X, Sanchez-Cardenas C, De la Vega-Beltran JL, Da Ros VG, Greer PA, Darszon A, Krapf D, Salicioni AM, Cuasnicu PS, Visconti PE (2016) The tyrosine kinase FER is responsible for the capacitation-associated increase in tyrosine phosphorylation in murine sperm. Development 143:2325

Baird A, Guest J, Willis B (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551

Baird AH, Edwards AJ, Guest JR, Harii S, Hatta M, Lachs L, Mera H, Sinniger F, Abrego D, Ben-Zvi O, Bronstein O, Cabaitan PC, Cumbo VR, Eyal G, Eyal-Shaham L, Feldman B, Figueiredo J, Flot J-F, Grinblat M, Heyward A, Hidaka M, Hirose M, Iguchi A, Isomura N, Kinzie RA, Kitanobo S, Kuba A, Levy O, Loya Y, Mezaki T, Mohamed AR, Morita M, Nojima S, Nozawa Y, Prasetia R, Puill-Stephan E, Ramirez-Portilla C, Rapuano H, Rosenberg Y, Sakai Y, Sakai K, Shlesinger T, Terraneo TI, Yakovleva I, Yamamoto HH, Yamazato K (2021a) A coral spawning calendar for Sesoko Station, Okinawa, Japan. Galaxea J Coral Reef Stud 24:41

Baird AH, Guest JR, Edwards AJ, Bauman AG, Bouwmeester J, Mera H, Abrego D, Alvarez-Noriega M, Babcock RC, Barbosa MB, Bonito V, Burt J, Cabaitan PC, Chang CF, Chavanich S, Chen CA, Chen CJ, Chen WJ, Chung FC, Connolly SR, Cumbo VR, Dornelas M, Doropoulos C, Eyal G, Eyal-Shaham L, Fadli N, Figueiredo J, Flot JF, Gan SH, Gomez E, Graham EM, Grinblat M, Gutierrez-Isaza N, Harii S, Harrison PL, Hatta M, Ho NAJ, Hoarau G, Hoogenboom M, Howells EJ, Iguchi A, Isomura N, Jamodiong EA, Jandang S, Keyse J, Kitanobo S, Kongjandtre N, Kuo CY, Ligson C, Lin CH, Low J, Loya Y, Maboloc EA, Madin JS, Mezaki T, Min C, Morita M, Moya A, Neo SH, Nitschke MR, Nojima S, Nozawa Y, Piromvaragorn S, Plathong S, Puill-Stephan E, Quigley K, Ramirez-Portilla C, Ricardo G, Sakai K, Sampayo E, Shlesinger T, Sikim L, Simpson C, Sims CA, Sinniger F, Spiji DA, Tabalanza T, Tan CH, Terraneo TI, Torda G, True J, Tun K, Vicentuan K, Viyakarn V, Waheed Z, Ward S, Willis B, Woods RM, Woolsey ES, Yamamoto HH, Yusuf S (2021b) An Indo-Pacific coral spawning database. Sci Data 8:35

Baro GC, Ritagliati C, Stival C, Luque GM, Gentile I, Buffone MG, Krapf D (2020) Everything you ever wanted to know about PKA regulation and its involvement in mammalian sperm capacitation. Mol Cell Endocrinol 518:110992

Barott KL, Helman Y, Haramaty L, Barron ME, Hess KC, Buck J, Levin LR, Tresguerres M (2013) High adenylyl cyclase activity and in vivo cAMP fluctuations in corals suggest central physiological role. Sci Rep 3:1379

Bicka M, Black C, Ghanaeian A, Joachimiak E, Osinka A, Majhi S, Konopka A, Bulska E, Bui KH, Wloga D (2023) Heterogeneity of radial spokes structural components and associated enzymes in *Tetrahymena* cilia. Cold Spring Harbor Laboratory, Cold Spring Harbor

Birkhead TR, Pizzari T (2002) Postcopulatory sexual selection. Nat Rev Genet 3:262

Bradley D, Beltrao P (2019) Evolution of protein kinase substrate recognition at the active site. PLoS Biol 17:e3000341

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R (2004) Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). Behav Ecol Sociol 56:65

Chen C, Ha BH, Thevenin AF, Lou HJ, Zhang R, Yip KY, Peterson JR, Gerstein M, Kim PM, Filippakopoulos P, Knapp S, Boggon TJ, Turk BE (2014) Identification of a major determinant for serine–threonine kinase phosphoacceptor specificity. Mol Cell 53:140

Durek P, Schudoma C, Weckwerth W, Selbig J, Walther D (2009) Detection and characterization of 3D-signature phosphorylation site motifs and their contribution towards improved phosphorylation site prediction in proteins. BMC Bioinform 10:117

Fitzpatrick JL (2020) Sperm competition and fertilization mode in fishes. Philos Trans R Soc Lond B 375:20200074

Fujita A, Nakamura K, Kato T, Watanabe N, Ishizaki T, Kimura K, Mizoguchi A, Narumiya S (2000) Ropporin, a sperm-specific

binding protein of rhophilin, that is localized in the fibrous sheath of sperm flagella. J Cell Sci 113(Pt 1):103
Gaillard AR, Diener DR, Rosenbaum JL, Sale WS (2001) Flagellar radial spoke protein 3 is an A-kinase anchoring protein (AKAP). J Cell Biol 153:443
Gaino E, Bo M, Boyer M, Scoccia F (2008) Sperm morphology in the black coral *Cirrhipathes* sp. (Anthozoa, Antipatharia). Invertebr Biol 127:249
Glass BH, Ashey J, Okongwu AR, Putnam HM, Barott KL (2023) Characterization of a sperm motility signalling pathway in a gonochoric coral suggests conservation across cnidarian sexual systems. Proc Biol Sci 290:20230085
Hagedorn M, Carter VL, Lager CVA, Camperio Ciani JF, Dygert AN, Schleiger RD, Henley EM (2016) Potential bleaching effects on coral reproduction. Reprod Fertil Dev 28:1061
Hayashibara T, Shimoike K, Kimura T, Hosaka S, Heyward A, Harrison P, Kudo K, Omori M (1993) Patterns of coral spawning at Akajima Islands, Okinawa, Japan. Mar Ecol Prog Ser 101:253
Hornbeck PV, Chabra I, Kornhauser JM, Skrzypek E, Zhang B (2004) PhosphoSite: a bioinformatics resource dedicated to physiological protein phosphorylation. Proteomics 4:1551
Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res 43:D512
Huang K, Diener DR, Rosenbaum JL (2009) The ubiquitin conjugation system is involved in the disassembly of cilia and flagella. J Cell Biol 186:601
Inaba K (2011) Sperm flagella: comparative and phylogenetic perspectives of protein components. Mol Hum Reprod 17:524
Inaba K, Morisawa S, Morisawa M (1998) Proteasomes regulate the motility of salmonid fish sperm through modulation of cAMP-dependent phosphorylation of an outer arm dynein light chain. J Cell Sci 111:1105
Inaba K, Kagami O, Ogawa K (1999) Tctex2-related outer arm dynein light chain is phosphorylated at activation of sperm motility. Biochem Biophys Res Commun 256:177
Inoue J, Satoh N (2019) ORTHOSCOPE: an automatic web tool for phylogenetically inferring bilaterian orthogroups with user-selected taxa. Mol Biol Evol 36:621
Izumi H, Marian T, Inaba K, Oka Y, Morisawa M (1999) Membrane hyperpolarization by sperm-activating and -attracting factor increases cAMP level and activates sperm motility in the ascidian *Ciona intestinalis*. Dev Biol 213:246
Kitanobo S, Toshino S, Morita M (2022) Genetic variation in released gametes produces genetic diversity in the offspring of the broadcast spawning coral *Acropora tenuis*. Sci Rep 12:5026
Knowlton N (2001) The future of coral reefs. Proc Natl Acad Sci USA 98:5419
Kreegipuu A, Blom N, Brunak S, Järv J (1998) Statistical analysis of protein kinase specificity determinants. FEBS Lett 430:45
Leclerc P, Kopf GS (1999) Evidence for the role of heterotrimeric guanine nucleotide-binding regulatory proteins in the regulation of the mouse sperm adenylyl cyclase by the egg's zona pellucida. J Androl 20:126
Leinbach SE, Speare KE, Rossin AM, Holstein DM, Strader ME (2021) Energetic and reproductive costs of coral recovery in divergent bleaching responses. Sci Rep 11:23546
Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10:228
Locatello L, Pilastro A, Deana R, Zarpellon A, Rasotto MB (2007) Variation pattern of sperm quality traits in two gobies with alternative mating tactics. Funct Ecol 21:975
Long H, Wang Q, Huang K (2015) Ciliary/flagellar protein ubiquitination. Cells 4:474
Loya Y, Sakai K (2008) Bidirectional sex change in mushroom stony corals. Proc Biol Sci 275:2335
Loza-Huerta A, Vera-Estrella R, Darszon A, Beltrán C (2013) Certain *Strongylocentrotus purpuratus* sperm mitochondrial proteins co-purify with low density detergent-insoluble membranes and are PKA or PKC-substrates possibly involved in sperm motility regulation. Biochim Biophys Acta Gen Subj 1830:5305
Mizrahi R, Breitbart H (2014) Mitochondrial PKA mediates sperm motility. Biochim Biophys Acta Gen Subj 1840:3404
Morita M, Nishikawa A, Nakajima A, Iguchi A, Sakai K, Takemura A, Okuno M (2006) Eggs regulate sperm flagellar motility initiation, chemotaxis and inhibition in the coral *Acropora digitifera*, *A. gemmifera* and *A. tenuis*. J Exp Biol 209:4574
Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K, Sakai K, Suzuki A (2010) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote 18:103
Nakajima A, Morita M, Takemura A, Kamimura S, Okuno M (2005) Increase in intracellular pH induces phosphorylation of axonemal proteins for activation of flagellar motility in starfish sperm. J Exp Biol 208:4411
Niinae T, Imami K, Sugiyama N, Ishihama Y (2021) Identification of endogenous kinase substrates by proximity labeling combined with kinase perturbation and phosphorylation motifs. Mol Cell Proteomics 20:100119
Nomura M, Inaba K, Morisawa M (2000) Cyclic AMP- and calmodulin-dependent phosphorylation of 21 and 26 kDa proteins in axoneme is a prerequisite for SAAF-induced motile activation in ascidian spermatozoa. Dev Growth Differ 42:129
Padilla-Gamiño JL, Weatherby TM, Waller RG, Gates RD (2011) Formation and structural organization of the egg–sperm bundle of the scleractinian coral *Montipora capitata*. Coral Reefs 30:371
Pandey AK, Ganguly HK, Sinha SK, Daniels KE, Yap GPA, Patel S, Zondlo NJ (2023) An inherent difference between serine and threonine phosphorylation: phosphothreonine strongly prefers a highly ordered, compact, cyclic conformation. ACS Chem Biol 18:1938
Pizzari T, Parker GA (2009) 6—Sperm competition and sperm phenotype. In: Birkhead TR, Hosken DJ, Pitnick S (eds) Sperm biology. Academic, London, pp 207–245
Portela P, Rossi S (2020) cAMP-PKA signal transduction specificity in *Saccharomyces cerevisiae*. Curr Genet 66:1093
R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Shiba K, Inaba K (2014) Distinct roles of soluble and transmembrane adenylyl cyclases in the regulation of flagellar motility in Ciona sperm. Int J Mol Sci 15:13192
Siebert S, Juliano CE (2017) Sex, polyps, and medusae: determination and maintenance of sex in cnidarians. Mol Reprod Dev 84:105
Soberg K, Jahnsen T, Rognes T, Skalhegg BS, Laerdahl JK (2013) Evolutionary paths of the cAMP-dependent protein kinase (PKA) catalytic subunits. PLoS ONE 8:e60935
Speer KF, Allen-Waller L, Novikov DR, Barott KL (2021) Molecular mechanisms of sperm motility are conserved in an early-branching metazoan. Proc Natl Acad Sci USA 118(48):e2109993118
Steiner SCC (1991) Sperm morphology of scleractinians from the Caribbean. Hydrobiologia 216:131
Su B, Bu Y, Engelberg D, Gelman IH (2010) SSeCKS/Gravin/AKAP12 inhibits cancer cell invasiveness and chemotaxis by suppressing a protein kinase C-Raf/MEK/ERK Pathway2. J Biol Chem 285:4578
Turner RM (2006) Moving to the beat: a review of mammalian sperm motility regulation. Reprod Fertil Dev 18:25
Vyklicka L, Lishko PV (2020) Dissecting the signaling pathways involved in the function of sperm flagellum. Curr Opin Cell Biol 63:154
Wallace CC (1999) Staghorn Corals of the World: a revision of the genus *Acropora*. CSIRO Publishing, Collingwood

Yang Z (1997) PAML: a program package for phylogenetic analysis by maximum likelihood. Bioinformatics 13:555
Yund PO (2000) How severe is sperm limitation in natural populations of marine free-spawners? Trends Ecol Evol 15:10
Zapata-Carmona H, Baron L, Zuniga LM, Diaz ES, Kong M, Drobnis EZ, Sutovsky P, Morales P (2019) The activation of the chymotrypsin-like activity of the proteasome is regulated by soluble adenyl cyclase/cAMP/protein kinase A pathway and required for human sperm capacitation. Mol Hum Reprod 25:587