Table 3 Search of *de novo* sequenced peptides of sea urchins in PepBank

Peptide	Origin	Function (possible)
DVKL	*A. lixula* – coelomic fluid	Antibiotic and anticancer
FLSY	*L. variegatus* – coelomic fluid	Phospholipase A_2 inhibitor
LDLR	*A. lixula* – coelomic fluid	Peptide in thrombin-liberated human/eglin (inhibitory activity towards human leukocyte elastase, cathepsin G, porcine pancreatic elastase and alpha-chymotrypsin)
LGSR	*E. lucunter* – coelomic fluid	Brain peptide for activation of prothoracic gland, factor VIII activity, toxin Tc61 (scorpion), human neutrophil NADPH oxidase factor 2
LLLL	*A. lixula* – coelomic fluid	Antimicrobial, neuroprotective
LPPP	*E. lucunter* – coelomic fluid	Dipeptidyl carboxypeptidase substrate
LVAL	*L. variegatus* – spines	Antiviral
PESL	*A. lixula* – coelomic fluid	Membrane-bound aminopeptidase P, protein tyrosine kinases

buffer or residual proteomic reagents (such as IAA, DTT, urea) were injected into the mass spectrometer, which causes the loss of extremely hydrophilic molecules. Nevertheless, several other peptides ($n = 22$) present in *E. lucunter* coelomic fluid could be identified. Some of them are related to marine known peptides, for instance PPVF, which is part of SpurS1, a neuropeptide from *Strongylocentrotus purpuratus* [10]. LGSR and LPPP are similar to neuropeptides that act on prothoracic glands or could be internal peptides of factor VIII, scorpion toxin Tc61, human neutrophil NADPH oxidase factor 2 or dipeptidyl carboxypeptidase.

Some of the peptides described in the present study could be aligned with others from sea urchins, which suggests the presence of bioactive peptides in Brazilian sea urchins. For example, antimicrobial peptides were aligned, indicating the presence of peptides with this function, which are present in many animals, especially marines, due to the environment rich in microorganisms [17, 18].

EDGAPDVSEVGGTFDQ, PSVGVVTLPTELPQ and QPMVVLCLVSTFDKSK were aligned with thymosin from *S. purpuratus* (Additional file 2). Thymosin acts on actin filament organization by sequestering actin monomers. Based on our previous studies on peptide toxins, it is our understanding that thymosin may be a source of cryptides that are bioactive peptides generated proteolytically from a non-precursor protein, by non-classical processing enzymes, and that display biological activities related or unrelated to the original protein [19]. The fragments of thymosin described herein were sequenced from secretions, not from cell lysates (e.g., intracellular). Moreover, these three peptides are derived from one single molecule, which indicates a possible 'natural' cleavage (not processing or artifact) generating peptides whose effects could be different from the original one (actin organization). For example, Schillaci et al. [11] have already reported that thymosin fragments, from *S. purpuratus*, possess antimicrobial activity. Nevertheless, complimentary experiments are necessary to evaluate this hypothesis.

Spines have also been reported as a potential source of bioactive molecules, since biological effects had been observed in spine extracts. Briefly, an inflammatory reaction in mice was described for the spine extract of *Echinometra lucunter*, confirming the presence of toxins in these structures [8]. Moreover, our group could identify a cathepsin B/X activity in spines, indicating the presence of enzymes for either chemical defense of the animal or tissue remodeling after injury, a frequent phenomenon observed in our collections [7].

In the same study from 2011, we have observed that spines are rich in small molecules, but no peptides are present [8]. These results were corroborated by the peptidomic approach, presented in the current work, in which we could not identify peptides in spines of *Echinometra lucunter.*

Lytechinus variegatus, on the other hand, presented peptides in both the spines and coelomic fluid. Although a few molecules have been described, biological effects have been related to this species, indicating the presence of active molecules. In 1963, Mendes et al. [20] reported an acetylcholine-like activity in the pedicellaria of *L. variegatus*, and that this molecule was deactivated when heated or submitted to NaOH, a situation typical for peptides or proteins. Nevertheless, this molecule (or molecules) has never been biochemically characterized.

In fact, several biological activities can be related to *L. variegatus* peptides obtained by *de novo* sequencing. FLSY, for example, is a phospholipase A_2 inhibitor and LVAL has antiviral activity. Moreover, AAHE was aligned with centrocin1a, an antimicrobial peptide from *Strongylocentrotus droebachiensis*; whereas VNDGTAALVVDNGSGFKV was aligned with papilosin, and GHNGY and MLGGAH were aligned with halocyntin [12, 13]. Papilosin and halocyntin are antimicrobial peptides obtained from hemocytes of the red sea squirt *Halocynthia papillosa*. These cells, responsible for phagocytosis and involved in the immune system, are also find in abundance in coelomic fluid of sea urchins [13]. Peptides similar to papilosin and halocyntin were identified in coelomic fluid of *L. variegatus*, which indicates the presence of antimicrobial molecules that are essential for maintenance of "sterile" conditions.

Arbacia lixula was the sea urchin with the largest number of identified peptides, both in spines and in coelomic fluid. From the latter, the sequenced peptides related to already described ones (not only marine) matched: an antimicrobial peptide, a peptide liberated by