# Marine Arthropods

Contributor: Juan Pablo Saucedo-Vázquez, Fernando Gushque, Nelson Santiago Vispo, Jenny Rodriguez, Marco Esteban Gudiño-Gomezjurado, Fernando Albericio, Markus P. Tellkamp, Frank Alexis

Peptide therapeutics play a key role in the development of new medical treatments. The traditional focus on endogenous peptides has shifted from first discovering other natural sources of these molecules, to later synthesizing those with unique bioactivities. Marine arthropods do not have an adaptive immune system, and therefore, they depend on the innate immune system to eliminate pathogens. Antimicrobial peptides (AMPs) with unique characteristics are a pivotal part of the defense systems of these organisms.

## 1. Introduction

Bioactive peptides have a broad spectrum of biological activities; from these, their immune signaling role stands out, due to their high selectivity and specificity for extracellular and intracellular target receptors. They are found in all living systems and play a decisive role in several physiological functions [1]. Given their vast variability and bioactive properties, peptides are considered to be pharmacologically ideal, in terms of safety, tolerability, and efficacy profiles, for the design of novel therapeutics for humans [2], thus increasing the wide family of traditional bioactive natural products from diverse sources such as plants, animals, and even bacteria [3][4]. Regulatory agencies have already approved around 100 pharmaceuticals in which a peptide serves as the active pharmaceutical ingredient (API) [5][6]. Of note, although cancer was traditionally the most important target for peptide-based drugs, recent years have witnessed the presence of bioactive peptides in practically all targets, with a special incidence in metabolic diseases and in radiopharmaceuticals [7].

Bioactive peptides can be isolated from natural sources. Such is the case of insulin, adrenocorticotropic hormone (ACTH), and calcitonin (canine or bovine pancreas, bovine or porcine pituitary glands, and salmon ultimobranchial gland), or they can be chemically synthesized or produced via fermentation [8]. In that regard, venoms from a wide range of animals, including cnidarians, echinoderms, mollusks, arthropods, and vertebrates, are now recognized sources of bioactive peptides for new potential therapeutics. In most cases, the isolation of peptides from natural sources is associated with the drug discovery process, which seeks to identify peptide sequences with the potential to become peptide drugs that can be produced synthetically or through genetic engineering [9]. Given their lack of stability in plasma and their poor ability to be transported through cell membranes as a result of the hydrophilicity inherent to their structures, native peptides face some limitations to becoming drugs. In this regard, the field of peptide chemistry is critical for the development of stable, active, and specific sequences of peptides with potential therapeutic uses. To this end, current strategies include the use of D-enantiomers, the modification of side chain length, the use of N-methylation or bulky residues, terminal capping, and cyclization to overcome protease degradation.

In addition, peptides are considered suitable templates for introducing chemical modifications, resulting in peptide mimetics that have also been approved by the corresponding regulatory agencies. Heterologous peptide discovery through synthetic library screening has resulted in the approval of only a few peptide drugs for clinical use. However, peptide analogues show improved properties over native peptides and they are the major chemical basis for new peptide drugs. In conclusion, advances in peptide chemistry and synthesis, and in drug delivery systems have brought about the success of peptide therapeutics and their conjugates.

Thus, the use of peptides with biological activity in therapeutics has growth significantly [10][11][12][13][14][15]. In this context, marine organisms are a major source of such molecules because their immune systems exert powerful action against several viral and bacterial diseases [16][17][18]. In this context, antimicrobial peptides (AMPs) play a key role in strengthening the immune systems of these creatures. The relevance of bioactive peptides is reflected in several reviews on AMPs in mammals [19], amphibians [20], fishes [21], plants [22], insects [23], echinoderms [24], marine arthropods [16][25][26], and penaeid shrimps [27][28]. Remarkable research has been conducted in this area [29]; therefore, researchers emphasize the synthetic chemistry of AMPs and their tentative medicinal applications. Researchers describe recent advances

regarding the molecular analysis of crustacean AMPs, concentrating on the antimicrobial mechanisms of these molecules against most pathogens in aquatic arthropods. These peptides not only serve to protect the organism against microbes (including Gram-positive and Gram-negative bacteria, yeast, and fungi) but also have antitumoral effects and mitogenic and immunoregulatory activity [30].

The immune response of crustaceans and chelicerates (Ecdysozoa, Arthropoda) is regulated on the external cuticle and the innate immune system, involving humoral and cellular effectors. Hemocytes are essential for the cellular immunity of these organisms, as they are involved in the storage and release of several defense molecules, including AMPs. Around 10% of animal-derived AMPs are from crustaceans, most of them (14 out of 15 families) having been identified in the decapod order (crabs, lobsters, crayfish, and several shrimp species) [31]. In marine crustaceans, the internal defenses rely on cellular and humoral responses (including pattern-recognition receptors/proteins, the production of reactive oxygen species, enzymatic cascades, clotting proteins, and AMPs) of the innate immune system that are mediated by blood cells or hemocytes [32]. The broad spectrum of biological activity shown by AMPs is a result of the wide variability of the subgroups and isoforms in a few families of these bioactive molecules. Indeed, current knowledge with regard to AMPs derived from marine crustaceans refers mostly to Decapoda, and thus does not represent the high diversity of sources [31] [33]. AMPs are defined as cationic, amphipathic, and single gene-encoded molecules (also expanded to anionic and multigene-encoded). Their structure predisposes them toward interaction with the bacterial membrane, and they are therefore a promising source of next-generation antibiotics.

In terms of amino acid composition, AMPs are classified into four groups: single-domain linear α-helical AMPs (e.g., armadillidin and homarin); single-domain AMPs containing Cys residues engaged in disulfide bonds (e.g., defensins, scygonadins, and anti-lipopolysaccharide factors (Alefs)); multi-domain or chimeric AMPs (e.g., penaedins, crustins, hyastatin, etc.); and unconventional AMPs, including multifunctional proteins and protein-derived fragments (e.g., histones and hemocyanin) [31]. Most AMPs display a broad spectrum of antimicrobial properties against bacteria, viruses, fungi, and protozoa. Penaedins and ALFs have potential uses in therapeutics due to a lower risk of resistance, a low toxicity, and potential immune modulator function. A major advantage of AMPs from marine crustaceans is their stability in the gastrointestinal tract environment.

## 2. Bioactive Peptides in the Subphylum Crustacea

### Diversity and Distribution

AMPs are ubiquitous defense molecules found in all life forms; unicellular organisms are thought to employ them during interspecific interactions, whereas multicellular life forms use them as part of their innate immune systems [34]. Despite the economic importance of many crustaceans, AMPs have not been extensively studied in these organisms, and research efforts continue to be channeled into identifying novel AMPs in a range of crustacean species [35]. Such efforts are likely to lead to the discovery of new AMPs, which will help to elucidate patterns in the evolution of these peptides in this animal group. To date, six AMPs have been identified in shrimp (order Decapoda, family Penaeidae) and 16 chemically distinct AMPs within the subphylum Crustacea (**Table 1**).

**Table 1.** Diversity of AMPs in Crustacea. Entries marked by an asterisk (*) in the third column were determined using the PHI-Blast alignment tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 1 March 2021).

| AMP | Characteristics | Sequence Affinity with Other Animal Taxa | Order | Family | Species | References |
|---|---|---|---|---|---|---|
| Bac-like | 6.5 kDa, Pro-rich, cationic | Fifth iteration, mostly Pro-rich bacterial peptides of unknown functions * | Decapoda | Carcinidae | *Carcinus maenas* | [36] |
| Callinectin | 3.7 kDa, Pro/Arg-rich, similar to arasins, cationic | No sequence similarity detected * | Decapoda | Portunidae | *Callinectes sapidus* | [37][38] |
| Astacidin-2 | 1.8 kDa, Pro/Arg-rich, cationic | Similar to arasin and arasin-like proteins from *Scylla* spp., *Hyas Araneus* * | Decapoda | Astacidae | *Pacifastacus leniusculus* | [39] |

| AMP | Characteristics | Sequence Affinity with Other Animal Taxa | Order | Family | Species | References |
|---|---|---|---|---|---|---|
| Armadillidin | 5.3 kDa, Gly-rich, cationic | No sequence similarity detected * | Isopoda | Armadillidiidae | *Armadillidium vulgare* | [40] |
| Homarin (CAP-1) | 4–6 kDa, putatively amphipathic α-helical, cationic | Similar to amphibian temporins | Decapoda | Nephropidae | *Homarus americanus* | [41] |
| Defensins | 6.8–7.2 kDa, contains three disulfide bonds, cationic | Shares six putatively homologous Cys residues with mammalian β-defensins | Decapoda | Palinuridae | *Panulirus japonicus* | [42] |
| Anti LPS factor | 7–11 kDa, contains a highly hydrophobic N-terminal region and the two Cys residues. The 3D structure of shrimp ALF consists of three α-helices and four-stranded β-sheets; the functional LPS-binding domain is a conserved cluster of positively charged residues within a β-hairpin located between two conserved Cys residues | Widely distributed in Crustacea * | Decapoda | Penaeidae | *Metapenaeus dobsoni*, *Penaeus* spp. (9) | [24][43][44] |
| | | | | Atyidae | *Neocaridina heteropoda* | |
| | | | | Palaemonidae | *Macrobrachium* spp. (3), *Palaemon carinicauda* | |
| | | | | Nephropidae | *Homarus americanus* | |
| | | | | Parastacidae | *Cherax quadricarinatus* | |
| | | | | Astacidae | *Pacifastacus leniusculus* | |
| | | | | Cambaridae | *Procambarus clarkii* | |
| | | | | Varunidae | *Eriocheir sinensis* | |
| | | | | Oregoniidae | *Cionoecetes opilio* | |
| | | | | Portunidae | *Charybdis* spp. (2), *Portunus* spp. (2), *Scylla* spp. (4) | |
| | | | Amphipoda | Hyaelellidae | *Hyalella azteca* | |
| | | | | Talitridae | *Trinorchestia longiramus* | |
| | | | Isopoda | Armadillidiidae | *Armadillidium* spp. (2) | |
| Scygonadin | 10.8 kDa, contains two Cys residues reminiscent of ALFs, anionic | Found only in *Scylla serrata* seminal fluid; shows similarities to ALFs, which might indicate a common evolutionary origin | Decapoda | Astacidae | *Scylla serrata* | [14] |
| *Scylla serrata* antimicrobial protein (SSAP) | 11.4 kDa, anionic | A Scygonadin homolog | Decapoda | Astacidae | *Scylla serrata* | [45] |

| AMP | Characteristics | Sequence Affinity with Other Animal Taxa | Order | Family | Species | References |
|---|---|---|---|---|---|---|
| Penaeidin | 4.7–7.2 kDa, cationic, N-terminal Pro/Arg-rich domain, C-terminal domain contains an amphipathic helix and two coils constrained by three disulfide bonds | Contains at least four subgroups found only in penaeidae shrimp | Decapoda | Penaeidae | *Penaeus* spp. (12) *Penaeus monoceros* | [16] |
| Crustin | 6–22 kDa, cationic, contains whey acidic protein (WAP) domain, further characterized by Gly-rich regions and conserved Cys residues | Found throughout Crustacea, and even in some hymenopterans * | Anostraca | Artemiidae | *Artemia salina* | [16][46] |
|  |  |  |  | Penaeidae | *Penaeus* spp. (6) |  |
|  |  |  |  | Atyidae | *Neocaridina heteropoda* |  |
|  |  |  |  | Palaemonidae | *Macrobrachium nipponense* |  |
|  |  |  |  | Pandalidae | *Pandalus japonicus* |  |
|  |  |  |  | Alvinocarididae | *Rimicaris exoculata* |  |
|  |  |  | Decapoda | Palinuridae | *Panulirus* spp. (2) |  |
|  |  |  |  | Nephropidae | *Homarus* spp. (2) |  |
|  |  |  |  | Parastasidae | *Cherax quadricarinatus* |  |
|  |  |  |  | Astacidae | *Pacifastacus leniusculus* |  |
|  |  |  |  | Lithodidae | *Paralithodes camtschaticus* |  |
|  |  |  |  | Oregoniidae | *Hyas araneus* |  |
|  |  |  |  | Portunidae | *Portunus pelagicus* *Scylla* spp. (3) |  |
| Hyastatin | 11.7 kDa, cationic, contains a Gly-rich region, Pro-rich domain, and Cys-rich domain | High sequence similarity with SpHyastatin | Decapoda | Oregoniidae | *Hyas araneus* *Cionoecetes opilio* | [47] |
|  |  |  |  | Portunidae | *Portunus trituberculatus* |  |
| SpHyastatin | 14.1 kDa, cationic, contains Pro-rich domain and Cys-rich domain | High sequence similarity with Hyastatin, but lacking a Gly-rich region | Decapoda | Portunidae | *Scylla paramamosain* | [47] |
| Arasin | 4.3–4.8 kDa, cationic, Pro- and Arg-rich | Similar to Astacidin-2 | Decapoda | Oregoniidae | *Hyas araneus* | [48] |
| Stylicin | 8.9 kDa, multidomain, N-terminal Pro/Arg-rich domain, C-terminal domain contains 13 Cys residues | Found only in Penaeidae. | Decapoda | Penaeidae | *Penaeus* spp. (4) *Penaeus japonicus* | [16] |

| AMP | Characteristics | Sequence Affinity with Other Animal Taxa | Order | Family | Species | References |
|---|---|---|---|---|---|---|
| Pellino-1-derived cationic antimicrobial prawn peptide | 8.0 kDa, β-sheet forming, cationic | Artificial, based on bioinformatic analyses of the structure of Pellino-1. | Decapoda | Palaemonidae | *Macrobrachium rosenbergii* | [49] |
| Histones or histone-derived peptides | Fi-Histidin: 2.9 kDa, enriched in Arg, Ala, Gly, Leu, Ser, cationic, α-helical structure H2A: 13.2 kDa, cationic H2B: 13.5 kDa, cationic | Highly conserved with great sequence similarity; antimicrobial action might be due to sequence at the N-terminus * | Decapoda | Penaeidae | *Penaeus* spp. (2) | [50][51] |
| Hemocyanin-derived peptides | C-terminus: 7.9–8.3 kDa, anionic, His-rich, α-helical structure Astacin-1: 1.9 kDa, cationic Predicted AMPs: 1.5–1.8 kDa | Highly conserved with great sequence similarity * | Decapoda | Penaeidae Astacidae Cambaridae | *Penaeus* spp. (2) *Pacifastacus leniusculus* *Procambarus clarkii* | [52][53][54] |
| Spgly-amp | 3.98 kDa, cationic, Gly-rich | Known from a single species with a hypothetical protein detected in *Portunus trituberculatus*, no sequence similarities detected in other Crustacean families *. | Decapoda | Portunidae | *Scylla paramamosain* | [55] |
| Scyreprocin | 9.1 kDa, cationic, Lys, Ala and Ser are the most common amino acids | Known from a single species with a hypothetical protein detected in *Portunus trituberculatus*, no sequence similarities detected in other Crustacean families *. | Decapoda | Portunidae | *Scylla paramamosain* | [56] |

Interestingly, not all of the AMPs are found in all the crustacean groups. While some are found in all Crustacean orders [44], hinting at an ancient origin of these molecules, others are limited to one or a few families, thereby suggesting a more recent evolution (**Table 1**). A good example of an ancient class of AMPs are the ALFs, which have undergone intense diversification. In contrast, penaeidins are a class of AMPs found in a single family of crustaceans, where they have diversified into four distinct subtypes. Therefore, there is a vast diversity of AMPs that could be elucidated through future bioinformatics and proteomic searches [57]. Clues as to the causes of the great diversification of some AMPs in the Crustacea subphylum can be found by looking at the amino acid composition. Many AMPs show a clear amino acid bias, being rich in Pro, Pro/Arg, or Gly. These amino acids cause the peptide to acquire certain structures and have a cationic nature (although a few anionic AMPs do exist). These properties predispose the peptides to interact in certain ways with the membrane of pathogens.

Thus, some AMPs may have resulted from nonsense mutations in large proteins that have domains rich in these amino acids. Pro-rich proteins are ubiquitous in eukaryotic genomes, and are thus common signaling molecules. In general, Pro/Arg-rich proteins that can provide a blueprint for AMPs are not as common, but they can be found throughout the genomes of several eukaryotic organisms and have diverse functions, such as C9ORF72 [58]. However, within the Crustacea subphylum, Pro-rich AMPs have a diverse evolutionary origin [59]. Gly-rich proteins are common throughout Eukaryotes and often have diverse functions [60]. Prior to the mutations that may have led to the creation of putative "pre-

AMPs", the gene in question would have had to undergo duplication to avoid the loss of functionality. These genes are molecular "hopeful monsters", resulting in new lineages of selfish genes that confer an important immune advantage to the individual, vis a vis population, in response to pathogens.

The proposed model for the diversification of other AMPs would also hold for the truncation of proteins that preserve entire domains, such as the single whey acidic protein (WAP) which contains eight well-conserved cysteine residues forming a four-disulfide core [31]. Truncation of proteins is followed by a nonsense mutation in one or more orthologs, of which only those with antibacterial properties confer an advantage to the animal. These novel AMPs may then have further multiplied through gene duplication and mutation. The observation that the antibacterial activity of AMPs is due to the properties of a certain group of amino acids suggests that the de novo synthesis of artificial AMPs could stem from the search of amino acid sequences with a similar bias or the search for motifs or domains with a heavy bias in the aforementioned amino acids [57].

## 3. Defense Mechanisms in Crustaceans

### 3.1. Hemocytes

Crustaceans belong to the phylum Arthropoda, and despite not having the sophistication of an adaptive immune system, they have a highly effective innate immune system to fight infections [61]. The first lines of crustacean defense are internal and external physical barriers. A strong external cuticle protects the body and the stomach, and the peritrophic membrane covers the midgut and hindgut [62]. The circulatory system of crustaceans is open; thus, the circulatory fluid, called hemolymph, flows freely through the circulatory system, which has open endings into the lacunal system (the hemocoel), which is in direct contact with tissues. The hemolymph has two components: cellular and humoral. The former is composed of hemocytes, the main immune effectors of the crustacean immune system. In contrast, several proteins, namely the respiratory pigment hemocyanin, the clotting factor, and several AMPs, among others, make up the humoral component. The crustacean hemolymph performs the functions of both the lymph and blood, and thus its biochemical and cellular components reflect the dual function of this tissue. For example, hemocyanin transports oxygen and participles in the immune response [63][64][65].

The innate immunity of crustaceans consists of a cellular and humoral response. The former involves the hemocytes, classified into three major types: hyaline cells (HCs), large granular cells (LGCs), and small-granular cells (SGCs). These cells exert diverse roles (Wu et al., 2019) as they interact with pathogens. HCs are involved in phagocytosis and coagulation, while SGCs participate in phagocytosis, nodule and capsule formation, and the release of the molecules of the prophenoloxidase (proPO) system and AMPs. LGCs store immune effector molecules, AMPs, and enzymes of proPO [66][67][68]. The proportions of each type of hemocyte in the hemolymph depend on various factors, such as molting [69][70]. Hemocytes are produced by hemopoietic tissues. In shrimp, these tissues are located as various epigastric lobules, mainly latero-dorsally to the cardiac stomach and in the proximal region of the maxillipeds. Hemopoietic hemocyte growth and differentiation is likely to be mediated by cytokines [66][71][72].

### 3.2. Cellular Reactions

Given the open nature of the shrimp circulatory system, hemocytes easily infiltrate tissues, and can respond in a localized way or migrate to specific tissues (gills, lymphoid organs, and connective tissue), where they trigger cellular reactions or release a range of compounds. The cellular reaction includes phagocytosis, which is performed mainly by HCs and SGCs, and is accompanied by degradative reactions via the generation of lytic enzymes or reactive oxygen or nitrogen species (ROS and RNS) generated during the respiratory burst [73]. In response to massive invasions of microorganisms or large microorganisms, hemocytes form cell aggregations called nodules. Denser cell aggregations made up of several layers of hemocytes are known as capsules. Sometimes, nodules or tubules of the hepatopancreas (invaded by *Vibrio* spp.) appear to be encapsulated by hemocytes. Melanin, a sticky and toxic dark pigment, is deposited in a very localized way in the central areas of nodules and capsules, or in wounds during the healing process. Melanin is generated by phenoloxidase, an enzyme that is produced via the activation of proPO system. The proPO system, one of the main immunoeffector responses of crustaceans, comprises a series of enzymatic cascade reactions [74] triggered by components of the cell walls of microorganisms, such as β-1,3-glucans from fungi, or lipopolysaccharide (LPS) and peptidoglycans from Gram-negative and Gram-positive bacteria, respectively [75]. Melanin formation generates intermediate reactive compounds (quinones and ROIS), which are more toxic than melanin. ProPO oxidase is regulated by several protease inhibitors, among them, pacifastina and α2-macroglobulin, produced by HCs. α2-macroglobulin inhibits serine proteases of the proPO cascade, and possibly pathogen proteases [76]. Hemocytes also participate in another cellular reaction, namely,

extracellular traps (ETs). This process involves the formation of DNA structures and AMPs to trap microorganisms. In the shrimp *Marsupenaeus japonicus*, ETs associated with c-type lysozyme have been described [77].

### 3.3. Other Immune Tissues

In addition to hemocytes, other tissues of the penaeid shrimp are involved in the immune response, including the heart reserve of phagocytes the podocytes of the gills, antennal gland, and the lymphoid organ (LO). The latter, which is near the hepatopancreas, receives hemolymph from the subgastric artery that forms a vascular plexus within this structure. The LO appears to filter viruses from the hemolymph [78]. Very strong hemocyte infiltration is detected in the LO, contributing to the immune functions of this organ. Many genes and molecules associated with hemocytes have been reported in the LO [78][79]. In this regard, a strong signal of penaeidin and α2-macroglobulin has been detected in the edges of tubules of the LO, in the same zone that participates in virus filtration. During infection, mainly that of viral etiology, nodular hyperplasia, known as LO spheroids (LOS) appear in the LO. These hyperplasic cells show a positive signal toward penaeidin and α2-macroglobulin [78]. It has been outlined that LOS are involved in the phagocytosis and destruction of foreign particles [80]. Terminal type C LOS exhibits an apoptosis signal, thus indicating the fate of engulfed material. Ectopic spheroids have also been detected in the connective tissues of other organs (stomach and buccal appendages).

Initially, there was some controversy regarding whether apoptosis as a response to viral infections in shrimp was a defense mechanism or a pathogenicity mechanism triggered by the virus [81]. The literature has reported that in the early stages of white spot syndrome virus (WSSV) infection, the virus exerts a mechanism to prevent host cells from entering apoptosis [82]. This observation thus indicates that apoptosis is a mechanism associated with the host's immune response.

### 3.4. Humoral Response

Humoral effectors involve the synthesis and secretion of immune proteins such as AMPs from hemocytes into the hemocoel. Part of the humoral response is the clotting system, which is stimulated by injuries and by the binding of microbial stimulants, such as β-1,3-D-glucans, to receptors on hemocytes. These cells release $Ca^{2+}$-dependent transglutaminases, which promote the polymerization of clotting proteins [83]. Finally, proteins derived from hemocyanin and hemocyanin-derived proteins have multiple functions in the hemolymph, from oxygen transport, antimicrobial activity, and agglutination, to playing a major role in the proPO system [63][64][65][83]. The multiple roles of hemocyanin-related proteins, as well as the diversity of AMP families, suggest that gene duplication and subsequent functional diversification are an important feature of the crustacean immune response. The open nature of the circulatory system of crustaceans, which is composed of vessel systems and a system of lacunae and sinuses surrounding the most important organs [84], facilitates the rapid migration and infiltration of hemocytes in different tissues, where they can then release their molecules in a localized manner.

### 3.5. Recognition of Foreign Molecules

Recognition molecules, including various types of lectins, galectins, β-1,3-glucanase-related protein, Toll-like receptors (TLR), Thioester-containing proteins (TEPs), scavenger receptors, Down syndrome adhesion molecules, and fibrinogen-related proteins, can circulate freely in the hemolymph or be bound to cell membranes. These molecules are able bind to pathogen-associated molecular patterns (PAMPs). Thus, signaling cascades in hemocytes are triggered, leading to the production and exocytosis of AMPs [85] and lysozymes, as well the activation of the proPO system.

## References

1. Chaplin, D.D. Overview of the Immune Response. J. Allergy Clin. Immunol. 2010, 2 (Suppl. 2), 125.

2. Fosgerau, K.; Hoffmann, T. Peptide therapeutics: Current status and future directions. Drug Discov. Today 2015, 20, 122–128.

3. Garcia-Gutierrez, E.; Mayer, M.J.; Cotter, P.D.; Narbad, A. Gut microbiota as a source of novel antimicrobials. Gut Microbes 2019, 10, 1–21.

4. Santos-Aberturas, J.; Vior, N.M. Beyond Soil-Dwelling Actinobacteria: Fantastic Antibiotics and Where to Find Them. Antibiotics 2022, 11, 195.

5. D'Aloisio, V.; Dognini, P.; Hutcheon, G.A.; Coxon, C.R. PepTherDia: Database and structural composition analysis of approved peptide therapeutics and diagnostics. Drug Discov. Today 2021, 26, 1409–1419.

6. Mullard, A. Targeted protein degraders crowd into the clinic. Nat. Rev. Drug Discov. 2021, 20, 247–250.

7. Muttenthaler, M.; King, G.; Adams, D.; Alewood, P. Trends in peptide drug discovery. Nat. Rev. Drug Discov. 2021, 20, 309–325.

8. Lau, J.L.; Dunn, M.K. Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorg. Med. Chem. 2018, 26, 2700–2707.

9. Pennington, M.W.; Czerwinski, A.; Norton, R.S. Peptide therapeutics from venom: Current status and potential. Bioorg. Med. Chem. 2018, 26, 2738–2758.

10. Mookherjee, N.; Anderson, M.A.; Haagsman, H.P.; Davidson, D.J. Antimicrobial host defence peptides: Functions and clinical potential. Nat. Rev. Drug Discov. 2020, 19, 311–332.

11. Shaer, A.; Musaimi, O.A.; Albericio, F.; de la Torre, B. 2019 FDA TIDES (Peptides and Oligonucleotides) Harvest. Pharmaceuticals 2020, 13, 40.

12. de la Torre, B.G.; Albericio, F. The Pharmaceutical Industry in 2019. An Analysis of FDA Drug Approvals from the Perspective of Molecules. Molecules 2020, 25, 745.

13. Ongey, L.; Pflugmacher, S.; Neubauer, P. Bioinspired Designs, Molecular Premise and Tools for Evaluating the Ecological Importance of Antimicrobial Peptides. Pharmaceuticals 2018, 11, 68.

14. Wang, L.; Dong, C.; Li, X.; Han, W.; Su, X. Anticancer potential of bioactive peptides from animal sources (Review). Oncol. Rep. 2017, 38, 637–651.

15. Sable, R.; Parajuli, P.; Jois, S. Peptides, Peptidomimetics, and Polypeptides from Marine Sources: A Wealth of Natural Sources for Pharmaceutical Applications. Mar. Drugs 2017, 15, 124.

16. Destoumieux-Garzón, D.; Rosa, R.D.; Schmitt, P.; Barreto, C.; Vidal-Dupiol, J.; Mitta, G.; Gueguen, Y.; Bachère, E. Antimicrobial peptides in marine invertebrate health and disease. Philos. Trans. R. Soc. B Biol. Sci. 2016, 371, 20150300.

17. Guzmán, F.; Wong, G.; Román, T.; Cárdenas, C.; Alvárez, C.; Schmitt, P.; Albericio, F.; Rojas, V. Identification of Antimicrobial Peptides from the Microalgae Tetraselmis suecica (Kylin) Butcher and Bactericidal Activity Improvement. Mar. Drugs 2019, 17, 453.

18. Bertrand, B.; Munoz-Garay, C. Marine Antimicrobial Peptides: A Promising Source of New Generation Antibiotics and Other Bio-active Molecules. Int. J. Pept. Res. Ther. 2019, 25, 1441–1450.

19. Dorin, J.R.; McHugh, B.J.; Cox, S.L.; Davidson, D.J. Chapter 30—Mammalian Antimicrobial Peptides; Defensins and Cathelicidins. In Molecular Medical Microbiology, 2nd ed.; Academic Press: Cambridge, MA, USA, 2015; pp. 539–565.

20. Patocka, J.; Nepovimova, E.; Klimova, B.; Wu, Q.; Kuca, K. Antimicrobial Peptides: Amphibian Host Defense Peptides. Curr. Med. Chem. 2019, 26, 5924–5946.

21. Shabir, U.; Ali, S.; Magray, A.R.; Ganai, B.A.; Firdous, P.; Hassan, T.; Nazir, R. Fish antimicrobial peptides (AMP's) as essential and promising molecular therapeutic agents: A review. Microb. Pathog. 2018, 114, 50–56.

22. Parisi, K.; Shafee, T.M.A.; Quimbar, P.; Weerden, N.L.v.d.; Bleackley, M.R.; Anderson, M.A. The evolution, function and mechanisms of action for plant defensins. Semin. Cell Dev. Biol. 2018, 88, 107–118.

23. Wu, Q.; Patočka, J.; Kuča, K. Insect Antimicrobial Peptides, a Mini Review. Toxins 2018, 10, 461.

24. Li, C.; Blencke, H.-M.; Haug, T.; Stensvåg, K. Antimicrobial peptides in echinoderm host defense. Dev. Comp. Immunol. 2015, 49, 190–197.

25. Huang, Y.; Ren, Q. Research progress in innate immunity of freshwater crustaceans. Dev. Comp. Immunol. 2020, 104, 103569.

26. Smith, V.J.; Dyrynda, E.A. Antimicrobial proteins: From old proteins, new tricks. Mol. Immunol. 2015, 68, 383–398.

27. Tassanakajon, A.; Rimphanitchayakit, V.; Visetnan, S.; Amparyup, P.; Somboonwiwat, K.; Charoensapsri, W.; Tang, S. Shrimp humoral responses against pathogens: Antimicrobial peptides and melanization. Dev. Comp. Immunol. 2018, 80, 81–93.

28. Tassanakajon, A.; Amparyup, P.; Somboonwiwat, K.; Supungul, P. Cationic Antimicrobial Peptides in Penaeid Shrimp. Mar. Biotechnol. 2010, 13, 487–505.

29. Matos, M.; Rosa, R.D. On the silver jubilee of crustacean antimicrobial peptides. Rev. Aquac. 2022, 14, 594–612.

30. Pfalzgraff, A.; Brandenburg, K.; Weindl, G. Antimicrobial Peptides and Their Therapeutic Potential for Bacterial Skin Infections and Wounds. Front. Pharmacol. 2018, 9, 281.

31. Rosa, R.D.; Barracco, M.A. Antimicrobial peptides in crustaceans. Invertebr. Surviv. J. 2010, 7, 262–284.

32. Mogensen, T.H. Pathogen Recognition and Inflammatory Signaling in Innate Immune Defenses. Clin. Microbiol. Rev. 2009, 22, 240–273.

33. Liu, M.-J.; Liu, S.; Liu, H.-P. Recent insights into hematopoiesis in crustaceans. Fish Shellfish Immunol. Rep. 2021, 2, 100040.

34. Mahlapuu, M.; Håkansson, J.; Ringstad, L.; Björn, C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agent. Front. Cell. Infect. Microbiol. 2016, 6, 194.

35. Yakovlev, A.; Lysøe, E.; Heldal, I.; Steen, H.; Hagen, S.B.; Clarke, J.L. Transcriptome profiling and in silico detection of the antimicrobial peptides of red king crab Paralithodes camtschaticus. Sci. Rep. 2020, 10, 1–10.

36. Schnapp, D.; Kemp, G.D.; Smith, V.J. Purification and characterization of a proline-rich antibacterial peptide, with sequence similarity to bactenecin-7, from the haemocytes of the shore crab, Carcinus maenas. Eur. J. Biochem. 1996, 240, 532–539.

37. Khoo, L.; Robinette, D.W.; Noga, E.J. Callinectin, an Antibacterial Peptide from Blue Crab, Callinectes sapidus, Hemocytes. Mar. Biotechnol. 1999, 1, 44–51.

38. Noga, J.; Stone, K.L.; Wood, A.; Gordon, W.L.; Robinette, D. Primary structure and cellular localization of callinectin, an antimicrobial peptide from the blue crab. Dev. Comp. Immunol. 2011, 35, 409–415.

39. Jiravanichpaisala, P.; Leea, S.Y.; Kima, Y.-A.; Andren, T.; Söderhäll, I. Antibacterial peptides in hemocytes and hematopoietic tissue from freshwater crayfish Pacifastacus leniusculus: Characterization and expression pattern. Dev. Comp. Immunol. 2007, 31, 441–455.

40. Herbinière, J.; Braquart-Varnier, C.; Grève, P.; Strub, J.-M.; Frère, J.; Dorsselaer, A.V.; Martin, G. Armadillidin: A novel glycine-rich antibacterial peptide directed against gram-positive bacteria in the woodlouse Armadillidium vulgare (Terrestrial Isopod, Crustacean). Dev. Comp. Immunol. 2005, 29, 489–499.

41. Battison, L.; Summerfield, R.; Patrzykat, A. Isolation and characterisation of two antimicrobial peptides from haemocytes of the American lobster Homarus americanus. Fish Shellfish Immunol. 2008, 25, 181–187.

42. Pisuttharachai, D.; Yasuike, M.; Aono, H.; Yano, Y.; Murakami, K.; Kondo, H.; Aoki, T.; Hirono, I. Characterization of two isoforms of Japanese spiny lobster Panulirus japonicus defensin cDNA. Dev. Comp. Immunol. 2009, 33, 434–438.

43. Gross, P.S.; Bartlett, T.C.; Browdy, C.L.; Chapman, R.W.; Warr, G.W. Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the Pacific White Shrimp, Litopenaeus vannamei, and the Atlantic White Shrimp, L. setiferus. Dev. Comp. Immunol. 2001, 25, 565–577.

44. Matos, M.; Schmitt, P.; Barreto, C.; Farias, N.D.; Toledo-Silva, G.; Guzmán, F.; Destoumieux-Garzón, D.; Perazzolo, L.M.; Rosa, R.D. Massive Gene Expansion and Sequence Diversification Is Associated with Diverse Tissue Distribution, Regulation and Antimicrobial Properties of Anti-Lipopolysaccharide Factors in Shrimp. Mar. Drugs 2018, 16, 381.

45. Yedery, R.D.; Reddy, K.V.R. Purification and characterization of antibacterial proteins from granular hemocytes of Indian mud crab, Scylla serrata. Acta Biochim. Pol. 2009, 56, 71–82.

46. Barreto, C.; Coelho, J.D.R.; Yuan, J.; Xiang, J.; Perazzolo, L.M.; Rosa, R.D. Specific Molecular Signatures for Type II Crustins in Penaeid Shrimp Uncovered by the Identification of Crustin-Like Antimicrobial Peptides in Litopenaeus vannamei. Mar. Drugs 2018, 16, 31.

47. Shan, Z.; Zhu, K.; Peng, H.; Chen, B.; Liu, J.; Chen, F.; Ma, X.; Wang, S.; Qiao, K.; Wang, K. The New Antimicrobial Peptide SpHyastatin from the Mud Crab Scylla paramamosain with Multiple Antimicrobial Mechanisms and High Effect on Bacterial Infection. Front. Microbiol. 2016, 7, 1140.

48. Stensvåg, K.; Haug, T.; Sperstad, S.V.; Rekdal, Ø.; Indrevoll, B.; Styrvold, O.B. Arasin 1, a proline–arginine-rich antimicrobial peptide isolated from the spider crab, Hyas Araneus. Dev. Comp. Immunol. 2008, 32, 275–285.

49. Ravichandran, G.; Kumaresan, V.; Arasu, M.V.; Al-Dhabi, N.A.; Ganesh, M.-R.; Mahesh, A.; Dhayalan, A.; Pasupuleti, M.; Arockiaraj, J. Pellino-1 derived cationic antimicrobial prawn peptide: Bactericidal activity, toxicity and mode of action. Mol. Immunol. 2016, 78, 171–182.

50. Patat, S.A.; Carnegie, R.B.; Kingsbury, C.; Gross, P.S.; Chapman, R.; Schey, K.L. Antimicrobial activity of histones from hemocytes of the Pacific white shrimp. Eur. J. Biochem. 2004, 271, 4825–4833.

51. Sruthy, S.; Nair, A.; Antony, S.P.; Puthumana, J.; Singh, I.B.; Philip, R. A histone H2A derived antimicrobial peptide, Fi-Histin from the Indian White shrimp, Fenneropenaeus indicus: Molecular and functional characterization. Fish Shellfish Immunol. 2019, 92, 667–679.

52. Destoumieux, D.; Bulet, P.; Strub, J.-M.; Dorsselaer, A.V.; Bachère, E. Recombinant expression and range of activity of penaeidins, antimicrobial peptides from penaeid shrimp. Eur. J. Biochem. 2001, 266, 335–346.

53. Lee, S.Y.; Lee, B.L.; Soderhall, K. Processing of an Antibacterial Peptide from Hemocyanin of the Freshwater Crayfish Pacifastacus leniusculus. J. Biol. Chem. 2003, 278, 7927–7933.

54. Yang, S.; Huang, H.; Wang, F.; Aweya, J.J.; Zheng, Z.; Zhang, Y. Prediction and characterization of a novel hemocyanin-derived antimicrobial peptide from shrimp Litopenaeus vannamei. Amino Acids 2018, 50, 995–1005.

55. Xie, Y.; Wan, H.; Zeng, X.; Zhang, Z.; Wang, Y. Characterization and antimicrobial evaluation of a new Spgly-AMP, glycine-rich antimicrobial peptide from the mud crab Scylla paramamosain. Fish Shellfish Immunol. 2020, 106, 384–392.

56. Yang, Y.; Chen, F.; Chen, H.-Y.; Peng, H.; Hao, H.; Wang, K.-J. A Novel Antimicrobial Peptide Scyreprocin From Mud Crab Scylla paramamosain Showing Potent Antifungal and Anti-biofilm Activity. Front. Microbiol. 2020, 1589.

57. Ravichandran, G.; Kumaresan, V.; Bhatt, P.; Arasu, M.V.; Al-Dhabi, N.A.; Arockiaraj, J. A Cumulative Strategy to Predict and Characterize Antimicrobial Peptides (AMPs) from Protein Database. Int. J. Pept. Res. Ther. 2016, 23, 281–290.

58. Farg, A.; Sundaramoorthy, V.; Sultana, J.M.; Yang, S.; Atkinson, R.A.; Levina, V.; Halloran, M.A.; Gleeson, P.A.; Blair, I.P.; Soo, K.Y.; et al. C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking. Hum. Mol. Genet. 2014, 23, 3579–3595.

59. Welch, G.; Li, W.; Hossain, M.A.; Separovic, F.; O'Brien-Simpson, N.M.; Wade, J.D. (Re) Defining the Proline-Rich Antimicrobial Peptide Family and the Identification of Putative New Members. Front. Chem. 2020, 8, 607769.

60. Bullard, R.; Sharma, S.R.; Das, P.K.; Morgan, S.E.; Karim, S. Repurposing of Glycine-Rich Proteins in Abiotic and Biotic Stresses in the Lone-Star Tick (Amblyomma americanum). Front. Physiol. 2019, 10, 1–13.

61. Hauton, C. The scope of the crustacean immune system for disease control. J. Invertebr. Pathol. 2012, 110, 251–260.

62. Martinez, P.; Falomir, M.P.; Gozalbo, D. Chitin: A Structural Biopolysaccharide with Multiple Applications; John Wiley & Sons: Hoboken, NJ, USA, 2014; pp. 1–10.

63. Lu, X.; Lu, H.; Guo, L.; Zhang, Z.; Zhao, X.; Zhong, M.; Li, S.; Zhang, Y. Cloning and Characterization of a Novel Hemocyanin Variant LvHMCV4 from Shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 2015, 46, 398–405.

64. Petit, V.; Jean-Luc, R.; Alain, B.; Chantal, C.; Chakib, D.; Jean, P.; Christophe, G.; Evelyne, B.; Joëlle, D.; Delphine, D.-G.; et al. A hemocyanin-derived antimicrobial peptide from the penaeid shrimp adopts an alpha-helical structure that specifically permeabilizes fungal membranes. Biochim. Biophys. Acta BBA 2016, 1860, 557–568.

65. Fan, J.; Li, X.; Lu, H.; Lin, R.; Aweya, J.J.; Zhang, Y. N-terminal diversity of Litopenaeus vannamei hemocyanin and immunity. Mol. Immunol. 2019, 112, 360–368.

66. Lin, X.; Söderhäll, I. Crustacean hematopoiesis and the astakine cytokines. Blood 2011, 117, 6417–6424.

67. Sanfratello, P.M.; Celi, M.; Vazzana, M. Hemocyte types and some plasmatic properties of two edible crabs Cancer borealis and Cancer pagurus. Invertebr. Surviv. J. 2015, 15, 195–202.

68. Wu, F.; Xie, Z.; Yan, M.; Li, Q.; Song, J.; Hu, M.; Wang, Y. Classification and characterization of hemocytes from two Asian horseshoe crab species Tachypleus tridentatus and Carcinoscorpius rotundicauda. Sci. Rep. 2019, 9, 1–10.

69. Jayasree, S.; Keerthana, C.K.; Anjali Menon, K.; Bhasura, K.; Rahna, S.; Reeha Mashirin, K.; Nidhila, M.; Akshaya, V.; Greeshma, M. Study on the effect of environmental stress on metabolic and immunological parameters in the haemolymph of Fenneropenaeus indicus H. Milne Edwards, 1837. Int. J. Fish. Aquat. Stud. 2017, 5, 490–497.

70. Day, R.D.; Fitzgibbon, Q.P.; Gardner, C. The impact of holding stressors on the immune function and haemolymph biochemistry of Southern Rock Lobsters (Jasus edwardsii). Fish Shellfish Immunol. 2019, 89, 660–671.

71. Braak, B.T.v.d.; Botterblom, M.H.A.; Liu, W.; Taverne, N.; Knaap, W.P.W.v.d.; Rombout, J.H.W.M. The role of the haematopoietic tissue in haemocyte production and maturation in the black tiger shrimp (Penaeus monodon). Fish. Shellfish. Immunol. 2002, 12, 253–272.

72. Söderhäll, I. Crustacean hematopoiesis. Dev. Comp. Immunol. 2016, 58, 129–141.

73. Muñoz, M.; Vandenbulcke, F.; Saulnier, D.; Bachère, E. Expression and distribution of penaeidin antimicrobial peptides are regulated by haemocyte reactions in microbial challenged shrimp. Eur. J. Biochem. 2002, 269, 2678–2689.

74. Lee, S.Y.; Soderhall, K. Early events in crustacean innate immunity. Fish Shellfish Immunol. 2002, 12, 421–437.

75. Cerenius, L.; Lee, B.L.; Soderhall, K. The proPO-system: Pros and cons for its role in invertebrate immunity. Trends Immunol. 2008, 29, 263–271.

76. Ponprateep, S.; Vatanavicharn, T.; Lo, C.F.; Tassanakajon, A.; Rimphanitchayakit, V. Alpha-2-macroglobulin is a modulator of prophenoloxidase system in pacific white shrimp Litopenaeus vannamai. Fish Shellfish Immunol. 2017, 62, 68–74.

77. Koiwai, K.; Alenton, R.R.R.; Kondo, H.; Hirono, I. Extracellular trap formation in kuruma shrimp (Marsupenaeus japonicus) hemocytes is coupled with c-type lysozyme. Fish Shellfish Immunol. 2016, 52, 206–209.

78. Rodríguez, J.; Ruiz, J.; Maldonado, M.; Echeverría, F. Immunodetection of hemocytes, peneidins and α2-macroglobulin in the lymphoid organ of white spot syndrome virus infected shrimp. Microbiol. Immunol. 2012, 56, 562–571.

79. Rusaini; Owens, L. Insight into the lymphoid organ of penaeid prawns: A review. Fish Shellfish Immunol. 2010, 29, 367–377.

80. Hasson, W.; Lightner, D.V.; Mohney, L.L.; Redman, R.M.; White, B.M. Role of lymphoid organ spheroids in chronic Taura syndrome virus (TSV) infections in Penaeus vannamei. Dis. Aquat. Org. 1999, 38, 93–105.

81. Khanobdee, K.; Soowannayan, C.; Flegel, T.W.; Ubol, S.; Withyachumnarnkul, B. Evidence for apoptosis correlated with mortality in the giant black tiger shrimp Penaeus monodon infected with yellow head virus. Dis. Aquat. Org. 2002, 48, 79–90.

82. Leu, J.H.; Lin, S.J.; Huang, J.Y.; Chen, T.C.; Lo, C.F. A model for apoptotic interaction between white spot syndrome virus and shrimp. Fish Shellfish Immunol. 2013, 34, 1011–1017.

83. Perdomo-Moralesa, R.; Montero-Alejoa, V.; Pererab, E. The clotting system in decapod crustaceans: History, current knowledge and what we need to know beyond the models. Fish Shellfish. Immunol. 2019, 84, 204–212.

84. Göpel, T.; Wirkner, C.S. The circulatory system of Penaeus vannamei Boone, 1931—Lacunar function and a reconsideration of the "open vs. closed system" debate. J. Morphol. 2020, 281, 500–512.

85. Li, S.; Lv, X.; Yu, Y.; Zhang, X.; Li, F. Molecular and Functional Diversity of Crustin-Like Genes in the Shrimp Litopenaeus vannamei. Mar. Drugs 2020, 18, 361.