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Original Research

Considerations for Predicting Peptides of the Insect Adipokinetic Hormone Ensemble Using Sequence Alignment

Simone König[1,*]

[1]Service Unit Proteomics, Medical Faculty, University of Münster, 48149 Münster, Germany
*Correspondence: koenigs@uni-muenster.de (Simone König)
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Abstract

Background: Adipokinetic hormones (AKHs) are metabolic regulators in insects, and many are still unknown. Bioinformatic analysis can assist entomologic research with predictions based on sequence similarity. **Methods:** AKHs are short peptides with specific sequence features, which were used together with the rules for preprohormone processing to interrogate National Center for Biotechnology Information (NCBI) genomic/transcriptomic data for novel putative AKH sequences by sequence alignment and similarity search. The structures of the signal peptide processing site and the dibasic processing site were evaluated in known and predicted AKHs. **Results:** 28 novel AKHs were predicted, as well as 8 peptides likely belonging to the AKH/corazonin-related peptide family. Furthermore, the presence of 42 known AKHs was suggested in 162 species, which have not yet been investigated experimentally for such peptide hormones. The dibasic processing site in known AKHs was almost exclusively sequence tag GKR. For the signal peptide processing site, residues A/C/G/S/T/V were mostly found in the –3-position and A/C/G/S in the –1-position. The results were assembled in a searchable archive of validated (from the literature), predicted (obtained by sequence search), and hypothetical (from combinatorial calculation) amino acid sequences, including taxonomy criteria and mass information. **Conclusions:** The archive is a snapshot of the current AKH landscape and useful as a reference and research tool. It allows grouping and sorting the entries for, e.g., order-specific sequences or peptide ion *m*/*z* values, and thus assists, among other purposes, chemical analysis with hypotheses for the identification of hormones in newly investigated species. The systematic comparison of the observed structures of the processing sites helps in future sequence evaluations for predicted AKHs.

Keywords: Arthropoda; insect; hormone; AKH; protein BLAST; sequence prediction

1. Introduction

Insect adipokinetic hormones (AKHs, for overview, see [1]) are synthesized in the neurohemal organs (corpora cardiaca, CC) and have important functions as metabolic regulators; their biologic properties have been extensively reviewed [1,2]. The majority of AKHs has been investigated in insects with very few examples from the other subphyla of the Arthropoda (consisting of: Hexapoda [Entognatha, Insecta—springtails, insects], Crustacea [Malacostraca, Maxillopoda, Branchiopoda—crabs, shrimps, woodlice, water fleas, barnacles, copepods], Myriapoda [Chilopoda, Diplopoda, Pauropoda, Symphyla—centipedes, millipedes], Chelicerata [Pycnogonida, Merostomata, Arachnida—scorpions, spiders, ticks, mites]) [1]. Globally, about 1.5 million, 5.5 million, and 7 million species of beetles, insects, and terrestrial arthropods, respectively, are estimated, with ~80% still thought to be undiscovered [3]. Thus, many AKHs remain to be found and investigated.

AKHs are peptides of mostly 8, occasionally 10 amino acid (AA) residues in length [1,4]. They are protected by N-terminal pyroglutamate (pQ) and amidation at the C-terminus, which is required for bioactivity [1,5]. Their sequence composition follows rules. Trp always occupies position 8; for positions 2 and 4, mostly hydrophobic residues have been found; position 3 varies only between Asn and Thr, and position 5 only between Ser and Thr. Position 6 has Pro in about two-thirds of all detected cases. Gly is often found in position 9. Fig. 1 gives an impression of the possible combinations of AA residues. For the analytical chemist tasked with the identification of an unknown peptide hormone in an insect CC extract, any prior knowledge can assist in de novo sequencing. Thus, based on these observations, as helpful tools for analysis, the MAPSP online application was programmed to determine possible sequence combinations for a given molecular weight [6], and a list of the 103 validated AKHs was assembled [4]. The focus of this list was on analytical parameters such as molecular weights and peptide ion *m*/*z* values. However, in order to understand the rules within the peptide ensemble, it is necessary to also consider taxonomic categories.

Thus, this work takes a fresh look at the published AKHs, whose number has increased to more than 120 different AA sequences, which were validated for AKH activity in hundreds of species (see reviews, e.g., for Scarabaeoidea [7], Cucujiformia [8], Lepidoptera [9], Diptera [10], Hemiptera [11], Polyphaga [12]). Bioinformatic analyses can provide hypothesis-generating informa-

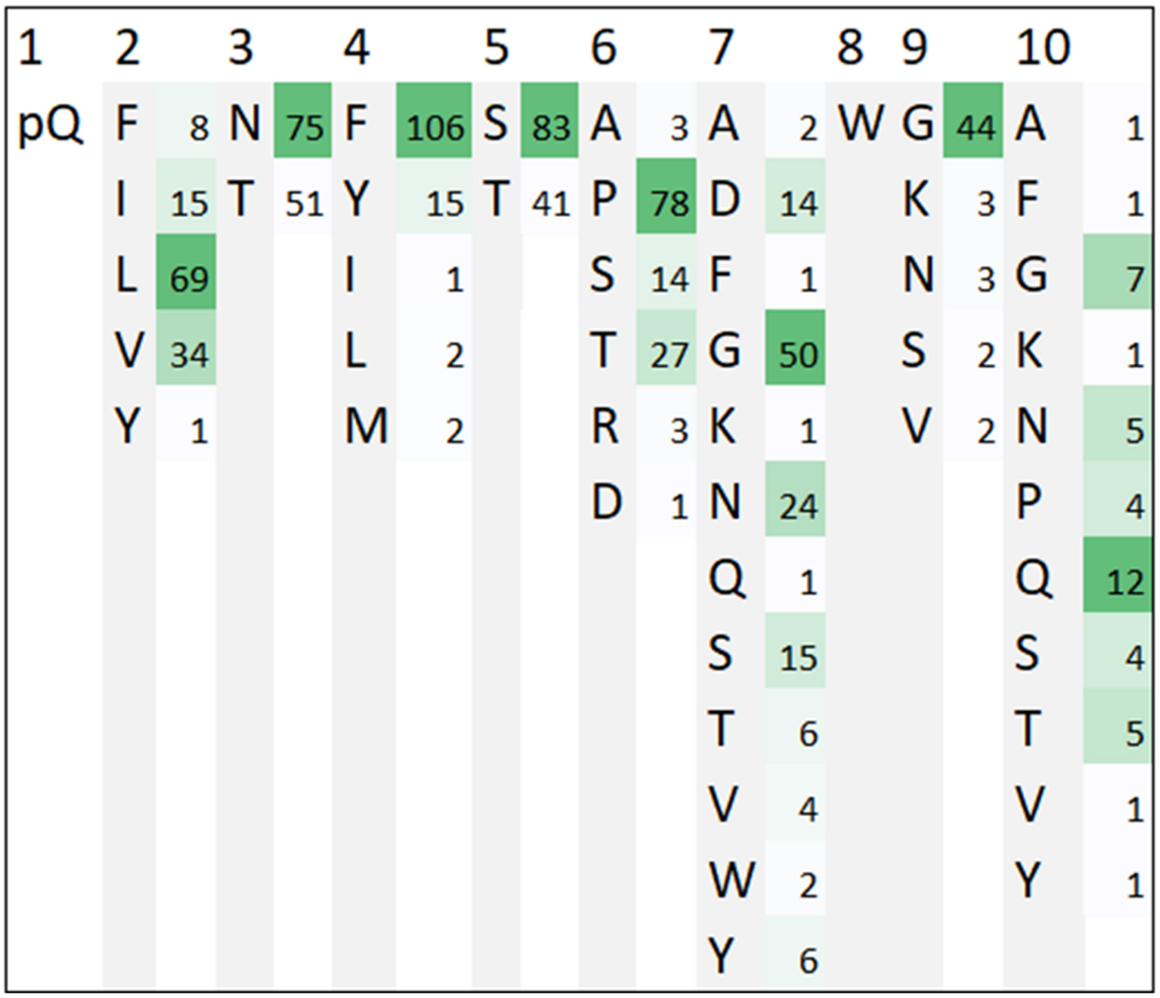

Fig. 1. Amino acid composition of known insect adipokinetic hormones. They are mostly octapeptides, occasionally decapeptides, with N-terminal pyroglutamate (pQ) and C-terminal amidation. The frequency of certain AA residues occurring in positions 1 to 10 is given and color-coded (most abundant: dark green).

tion about animals, which are not easily accessible in sufficient numbers for direct experimental study. While sequence predictions cannot replace the laboratory determination and validation of a suspected biological function, they can speed up the analytical process, as shown for cockroaches [13,14]. Although there is no guarantee that a predicted AKH is truly expressed or processed to the active hormone in an animal, knowledge of a possible peptide sequence from genomics or transcriptomics reduces the analysis effort for a particular sample from complicated de novo sequencing to a much simpler confirmation experiment [13,14]. In fact, Gäde and coworkers [15] conducted a bioinformatic study of AKH ligand and receptor gene evolution in Blattodea based on nucleotide collections and predicted nine putatively novel AKHs.

In the present study, in a different approach, AKH sequences for insects were extracted from the National Center for Biotechnology Information database (NCBI DB), the leading resource for protein and gene sequences, with the aim of getting a snapshot of the currently available information and potentially finding novel putative AKHs from genome assembly data, taking also into account protein processing rules.

In early post-translational processing, signal peptidases remove the signal peptide (SP) upstream from the hormone sequence (Fig. 2, Ref. [4]) [16,17]. Thereby, the (–3,–1)-rule has been established to predict the most likely cleavage site directly from the primary sequence, with positions –3 and –1 relative to the cleavage site being the most important ones [18,19,20]. Von Heijnes' original work [18] suggests small residues excluding Pro in positions –1 and –3 (–1-position: Ala, Ser, Gly, Cys, Thr, Gln; –3-position: non-aromatic, uncharged, unpolar AA residues). Subsequent cyclization of the N-terminal glutamine into pyroglutamate by glutaminyl cyclase also appears to be an early step in the AKH biosynthetic pathway [16]. Prohormone convertases then require dibasic sites such as Lys-Arg or Arg-Arg for the further liberation of the

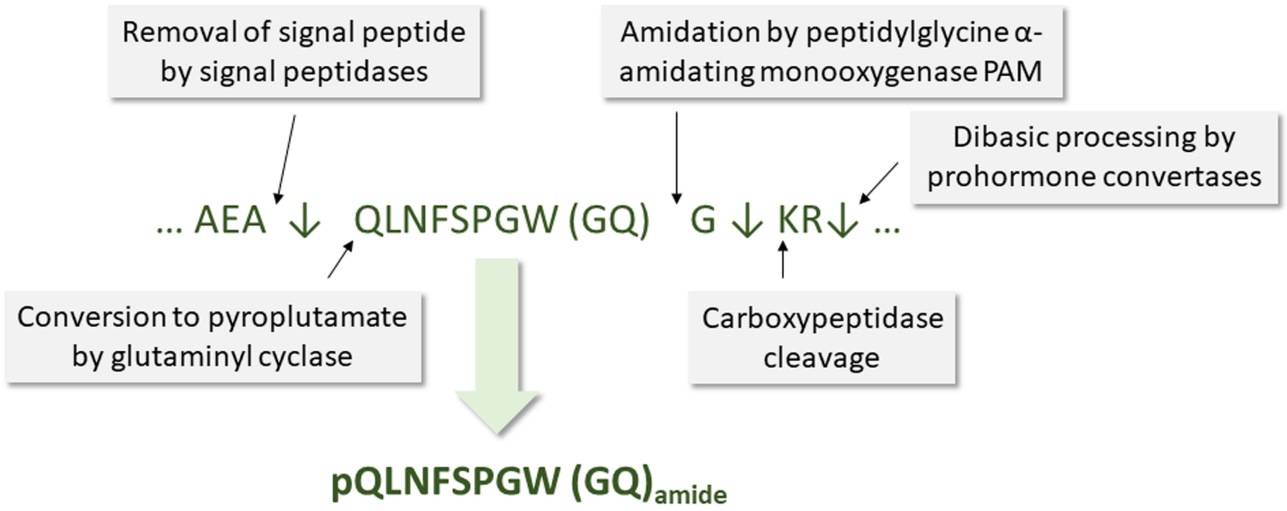

Fig. 2. Processing steps to form the mature AKH from the preprohormone. Exemplarily, a peptide sequence is given based on the most abundant residues in the respective positions in known AKHs (see Fig. 1; incidentally, the octapeptide corresponds to AKH Panbo-RPCH, see **Supplementary Table 1**, entry 50 [4]). The brackets indicate octa- and decapeptides. Arrows point to cleavage sites. Please note that the mature AKH is protected by pyroglutamate (pQ) at the N-terminus and is amidated at the C-terminus.

AKH sequence [16,21]. Importantly, a Gly residue must be present at the C-terminal side of the residue α-amidated in the product peptide to allow amidation by peptidyl-glycine α-amidating monooxygenase PAM [5]. Further, the formation of dimeric prohormones was demonstrated in locusts [16], but is not considered as a search parameter in the present approach, neither are post-translational modifications [22].

2. Methods

DB entries accessible through NCBI vary from short sequences of the mature peptides such as Grybi-AKH (**Supplementary Table 1**, entry 313 [11]) to information about fragmental or full-length protein sequences containing this particular stretch of AA residues (e.g., KAK7790124.1, entry 318, for a "hypothetical protein" from *Gryllus longicercus*). The entries are mostly derived from translated genome assembly and annotation data and are labeled as "uncharacterized" or "hypothetical predicted" proteins, but sometimes they also originate from protein and peptide sequencing. If their function has been elucidated by bioinformatic or experimental assignment, they may have been labeled "adipokinetic (pro)hormone", "red-pigment-concentrating (pro)hormone-like", or "hypertrehalosaemic (pro)hormone". The present work mines these data using the NCBI blastp procedure (Protein BLAST) in the nr (non-redundant protein sequences) DB of taxid 6960 (Insects) and, for completeness' sake, in the superordinate taxid 6656 (Arthropoda).

In order to access potential AKHs in the vast body of archived sequences, sequential experiments using the NCBI blastp suite (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed April 2026) were performed using a text file containing the sequences of the known and validated AKHs as compiled from the latest reviews and publications (for references, see **Supplementary Table 1**). The discovery of the individual AKHs was not the focus here, but it can be traced from the given references. Peptide modifications were not considered in the search procedure. Please be aware that while it is clear that mature AKHs are terminally protected, for alignment analysis, unmodified AAs have to be used. This process detected and exported not only identical, but also similar sequences, ensuring wide coverage and eliminating the need for a search with all possible AA combinations (more than 7200 based on the AA residues shown in Fig. 1), which would unduly occupy search capacity at NCBI and produce tens of thousands of identical entries in the results lists. A text file meeting the blastp submission criteria was created for the purpose, containing the individual AKH sequences extended by AA residues GKR for the dibasic processing site (example: QFNFSAGW GKR for Cirba-AKH, **Supplementary Table 1**, entry 2 [12]). BLAST analysis also finds sequences extended by GRR when GKR is requested in the bait file, because, by design, it reports a choice of similar sequences for each input sequence. The results of the BLAST search for each AKH were downloaded as separate dump files (function of the BLAST results website) and combined in Excel. Duplicates and hits not meeting the requirements (see Introduction) were removed manually using the Excel sorting function. Information about the SP cleavage site was obtained by calling up entries individually in NCBI and reading off the three AA residues N-terminal to the sequence region of the putative AKH. It was not useful to include examples for the cleavage site of the signal peptide already during the BLAST search (by submitting sequences in the form, e.g.,

AAA QFNFSAGW GKR), because as a result, the quality of AKH core sequence recognition suffered considerably. The manual approach was necessary to answer the following questions: (1) What are the SP sites in validated AKHs? (2) How do they compare to the known rules for these sites?, and (3) How can this knowledge be applied to the BLAST results in order to short-list them further? This procedure was a necessary step in understanding the structure of the data.

Sequences for the known AKHs were added to the results list for comparison, and the tables were sorted alphabetically (**Supplementary Table 1** "Insects" and **Supplementary Table 4** "Arthropoda"). In **Supplementary Table 1**, sequences are represented by a single DB entry; multiple accession numbers (e.g., for isoforms or fragments) are supplied in **Supplementary Table 4**. DB entries for the search in Arthropoda are, for a further overview, additionally listed in the context of all theoretically possible sequence variations (**Supplementary Table 5** "With combinations"), which were generated with Visual Basic based on the AA residue distribution in known AKHs (see Fig. 1). Peptide mass and ion values were supplied for added benefit.

3. Results and Discussion

Any prior knowledge helps the analytical chemist with the identification of an unknown peptide hormone in an insect CC extract. This includes special ionisation and gas phase fragmentation behaviour in mass spectrometry experiments [23]. For instance, the presence of Pro in position 6 causes very characteristic cleavage patterns upon collision-induced dissociation and, additionally, the Trp immonium ion can serve as a marker [4]. Furthermore, the special rules for AKH sequences allow correlating peptide and AA residue masses, spectral ion profiles, and sequence hypotheses [6]. Thus, for reference, a list of the theoretically possible AKH sequence combinations for octapeptides, calculated based on the presently available information about AKH AA composition (see Fig. 1), is provided in **Supplementary Table 5** along with their molecular weights and ion *m*/*z* values. A calculation tool for this purpose was published earlier [4].

Supplementary Table 1 lists in 443 entries the results of the BLAST search in insects together with the known AKHs in alphabetical sequence order. Entries are discussed below in detail. It is strongly advised to keep **Supplementary Table 1** open during reading for better understanding.

3.1 Outlier

Irrelevant search hits were removed during data curation; a few questionable entries have, however, been left in the list to be discussed. Sequence number 1 (QFGYGPRW) of a "hypothetical protein" obtained from the draft genome of the Japanese rhinoceros beetle *Trypoxylus dichotomus* (Coleoptera, Polyphaga, Scarabaeidae) has Gly in positions 3 and 5 of the 8-AA AKH core sequence, which does not agree with the sequence rules found for AKHs so far (see Fig. 1: Asn or Thr in position 3, Ser or Thr in position 5). Still, its processing sites look good. Thus, entry 1 in **Supplementary Table 1** was flagged in yellow colour, but left in the list as both a reminder of potential future surprises and a control factor regarding the limitations of AKH sequence screenings.

So far, for the beetle family Scarabaeidae, Melme-CC, Euoin-AKH, Cirba-AKH, Scade-CC-I/II, Trifa-CC, Oniay-CC, Dorpa-AKH, Lucce-AKH, Ampso-AKH-I/II, and Ontbi-AKH have been validated [7] (for their sequences, see **Supplementary Table 1**, entries 142, 14, 2, 4, 6, 16, 443, 345, 5, 343, 141, 344). Also, rather rare modifications were detected in beetles such as Tyr in position 2 in Oniay-CC (pQYNFSTGWam, entry 443), making this AKH the last entry of the alphabetically sorted canon of known AKHs shown in **Supplementary Table 1** [7,12,22].

3.2 Processing Site Structure

The known AKHs, for which the sequence of the SP cleavage site could be determined (in DB entries representing only mature peptides, this was not possible), show Ala, Cys, Ser, Gly, Thr, Val in the –3-position and Ala, Cys, Gly, Ser in the –1-position of the cleavage site, fully agreeing with the (–3,–1)-rule [18] (for SP sequences, see **Supplementary Tables 1,4**). Few exceptions from this rule have been observed, and only when no better cleavage site was available in the vicinity [18].

Entry 158 from an uncharacterized dipteran protein supplied by automated computational analysis is suspicious, because the signal processing site contains Pro in position –3, and Asp in position –1, which are typically excluded (see Introduction) [18]. Thus, it was marked. The sequence QLTFSPGWGQ of entry 158 corresponds to Antya-AKH (entry157), which was described in Lepidoptera [9].

Similar caution is suggested for entry 181 having unpolar Leu at the –3-position. The sequence for this entry (QLTFSSGW) has, however, been predicted in five more lepidopteran species (entries 176–180) with well-fitting SP sites. It corresponds to Piebr-AKH (entry 174), which has already been investigated in Lepidoptera and Hemiptera [9].

Ile at position –3 of the signal peptide cleavage site (entries 183, 203) is also rare, but that may not be critical, in particular, because sequence QLTFSSGWGN corresponding to Helze-HrTH from the corn earworm [9] (entry 182, Lepidoptera) is predicted in two more lepidopteran species with well matching SP sites (entries 184, 185). The situation is similar for sequence QLTFTPGW predicting Tabat-AKH from the horsefly (entry 201 [24]) in dipteran sandflies *Phlebotomus argentipes* (entry 203) and *Sergentomyia squamirostris* (entry 204), but also in net-spinning caddisfly *Arctopsyche grandis* (entry 205, Trichoptera).

Likewise, Thr is observed at the –1-position in entries 26 (Lepidoptera), 45 (Hymenoptera), 181 (Lepidoptera, see above) and 442 (Neuroptera). While Thr is common at the –3-position, it is not typical for the –1-position in AKHs (seen so far: A/C/G/S/T/V x A/C/G/S), but also not excluded by the (–3,–1)-rule [18].

All of these possible residues for the SP cleavage site have been detected in Diptera and Hemiptera and most of them in Blattodea, Coleoptera, Hymenoptera, Lepidoptera and Odonata, because from these orders the majority of the data were available. In fact, most entries for novel insect AKHs came from the Hymenoptera and Lepidoptera, followed by Diptera and Coleoptera (**Supplementary Tables 2** "Novel", **Supplementary Tables 3** "New in species"). The dibasic processing sites were exclusively GKR with the exception of a *Schistocerca* peptide (**Supplementary Table 4**, entries 244–246), where it was GRR.

3.3 AKH/Corazonin-Related Peptides

For the American cockroach, two different sequences have been validated as AKHs (Peram-CAH-I and Peram-CAH-II: pQVNFSPNWam and pQLTFTPNWam [24]). Entry 360 in **Supplementary Table 1** represents a 96 AA-long sequence for an AKH/corazonin-related peptide (ACP)-like protein from the cockroach *Periplaneta americana* (predicted by automated computational analysis and containing the sequence stretch ALA QVTFSRDWNA GKR). The finding of this ACP shows a weakness of the screening approach, because it also detects representatives of this entirely different hormonal system. ACPs are structurally intermediate between AKHs and corazonins, but each peptide family has its own receptors [1,25]. ACPs do not activate the AKH and corazonin receptors and vice versa [25]. The ACP signalling system was not always found in insects, indicating that it has been lost several times during arthropod evolution [25]. Although corazonins are also structurally related to AKHs [2], in contrast to ACPs, they are not selected in the screening process when using the above criteria.

The peptide pQVTFSRDWNAam, Rhopr-ACP (**Supplementary Table 1**, entry 357), has been identified as an ACP in the kissing bug *Rhodnius prolixus*, mosquitoes, and a few other insects [13,25,26]; thus, the 43 entries (358–400) for this sequence in **Supplementary Table 1** are likely also ACPs. There are 82 entries with this same octapeptide core structure differing only in positions 9 and 10 (KA – entry 355, KG – entry 356, NP – entries 401–409, SA – entry 410, SG – entries 411–425, TA – entries 428–433, TG – entries 434–436, TP – entries 437–439) with three entries flagged for their SP site composition (entry 414 for Gln at position –1, entry 354 for Asn and entry 377 for Ile at position –3). Entry 426 for pQVTFSRDWSPam (Lom-HrTH) was published as "inactive AKH" in locust [27].

Until the discovery of their receptors, ACPs were often miscategorized as AKH due to the very similar core structure. Although the AA combination QVTFSRDW appears to be unique for ACPs [25], it has yet to be proven that there are no exceptions. In particular, the Arg-Asp charge pair in positions 6 and 7 has been associated with ACPs [25], but it is also found in Bommo-AKH-III (pQITFSRDWSGam, entry 23 in **Supplementary Table 1** [28]) and is predicted in 24 species with this core sequence (entries 20–44). Negatively charged Asp in position 7, although special among the typically neutral AA residues in that position, has also been observed in several AKHs (Scade-CC-I, entry 4; Trica-AKH, entry 65; Melme-CC, entry 142; Rhopr-AKH, entry 189 [8,12,26]). More experimental data are needed for clarification. Thus, the search matches for potential ACPs were retained in the **Supplementary Tables**, but excluded from Table 1 (Ref. [4,7,8,9,10,11,12,13,14,24,28,29,30,31]) and Table 2 below, because the focus of the present work is on insect AKHs.

Table 1 presents the number of species in which known AKHs were predicted in addition to those already mentioned in the literature (total 162 species, **Supplementary Table 3**). Table 2 shows 28 predicted novel sequences for AKHs (66 entries, **Supplementary Table 2**).

3.4 Predicted Novel Peptides

The discussion below refers to the numbers for novel peptides in Table 2 and entries in **Supplementary Table 1**. It is strongly recommended to keep **Supplementary Table 1** open during reading for improved understanding.

Sequence 1 (QINYSPGW) differs from coleopteran Harax-AKH of the lady beetle (entry 19 [4]) by Pro for Thr in position 6 and was predicted for mayflies.

The following peptide compositions in the list resemble Bommo-AKH-III from the silk moth *Bombyx mori* (pQITFSRDWSGam, Lepidoptera, entry 23 [28]) in its core, but vary in positions 9 and 10. Bommo-AKH-III itself has been predicted for four more Lepidopterans (entries 24–27). While QITFSRDWTG has been suggested for 16 additional Lepidopteran species (entries 29–44), there are also results for AA residues MG (entry 21) and TC (entry 28) in positions 9 and 10 in Lepidoptera. There is one hit, respectively, for the aster leafhopper (Hemiptera, entry 20) and one for a field cricket (Orthoptera, entry 22) with KN and NA in these positions. Sequence 7 (QITFSTDWAQ) differs from this 8-AA core only by a Thr6 for Arg6 switch and was predicted for an ant (Hymenoptera, entry 45), as were sequences 8 (QLNFSTDWGQ, entry 67) and 9 (QLNFSTEW, entry 68).

Peptides 10 and 11, predicted for 13 species of Hymenoptera (entries 118–130), share the 8-AA core with Schgr-AKH-II (pQLNFSTGWam [12], predicted in 46 additional species of Coleoptera and Hymenoptera, entries 72–117) and resemble decapeptide Tenar-HrTH from the

Table 1. Sequences of validated AKH peptides as known from the latest literature and the number of predicted sequences in other species (summary of Supplementary Table 3; for individual hits, see there).

#	1	2	3	4	5	6	7	8	9	10	AKH	n	Predicted in order	Known in order
1	**Q**	I	N	F	T	P	N	**W**			Micvi-CC [24]	1	Coleoptera	Blattodea Coleoptera
2	**Q**	I	T	F	S	R	D	**W**	S	G	Bommo-AKH-III [28]	4	Lepidoptera	Lepidoptera
3	**Q**	I	T	F	S	T	G	**W**			Cimle-AKH [11]	1	Hymenoptera	Hemiptera
4	**Q**	L	N	F	S	P	G	**W**			Panbo-RPCH [29]	1	Hemiptera	Hemiptera Coleoptera Malacostraca, Decapoda
5	**Q**	L	N	F	S	P	N	**W**			Tenmo-HrTH [24]	2	Neuroptera Coleoptera	Coleoptera Hemiptera Blattodea Orthoptera
6	**Q**	L	N	F	S	P	S	**W**			Corpu-AKH [11]	1	Hemiptera	Hemiptera
7	**Q**	L	N	F	S	T	D	**W**			Trica-AKH [8]	1	Coleoptera	Coleoptera
8	**Q**	L	N	F	S	T	G	**W**			Schgr-AKH-II [12]	46	Hymenoptera	Orthoptera Coleoptera Hemiptera Hymenoptera
9	**Q**	L	N	F	T	P	N	**W**			Pyrap-AKH [24]	3	Coleoptera	Hemiptera Coleoptera Orthoptera
10	**Q**	L	N	Y	S	P	D	**W**			Melme-CC [12]	1	Coleoptera	Coleoptera
11	**Q**	L	T	F	S	P	D	**W**			Phote-HrTH [4]	5	Diptera	Diptera Orthoptera
12	**Q**	L	T	F	S	P	G	**W**			Glomo-AKH [4]	1	Diptera	Diptera Hemiptera
13	**Q**	L	T	F	S	P	N	**W**			Diptera novel [11]	3	Lepidoptera	Hemiptera Diptera
14	**Q**	L	T	F	S	P	S	**W**			Tippa-CC-II [10]	1	Diptera	Diptera Coleoptera
15	**Q**	L	T	F	S	S	G	**W**			Piebr-AKH [9]	6	Lepidoptera	Lepidoptera Hemiptera
16	**Q**	L	T	F	S	S	G	**W**	G	N	Helze-HrTH [9]	3	Lepidoptera	Lepidoptera
17	**Q**	L	T	F	S	S	G	**W**	G	Q	Manse-AKH-II [9]	2	Lepidoptera	Lepidoptera
18	**Q**	L	T	F	S	T	G	**W**			Dircl-AKH-II [11]	1	Hymenoptera	Lepidoptera Hemiptera
19	**Q**	L	T	F	S	T	G	**W**	G	N	Chipa-AKH [4]	2	Diptera	Lepidoptera
20	**Q**	L	T	F	S	T	G	**W**	G	Q	Ostnu-AKH [14]	1	Lepidoptera	Lepidoptera
21	**Q**	L	T	F	T	P	A	**W**			Anoga-AKH [10]	3	Diptera	Diptera
22	**Q**	L	T	F	T	P	G	**W**			Tabat-AKH [24]	3	Diptera Trichoptera	Diptera
23	**Q**	L	T	F	T	P	N	**W**			Peram-CAH-II [24]	5	Diptera Lepidoptera	Blattodea Coleoptera Lepidoptera Hemiptera Orthoptera
24	**Q**	L	T	F	T	P	S	**W**			Aedae-AKH [10]	4	Orthoptera Diptera	Diptera Megaloptera
25	**Q**	L	T	F	T	P	V	**W**			Novel [10]	2	Diptera Siphonaptera	Diptera
26	**Q**	L	T	F	T	S	S	**W**			Hipes-AKH-I [9]	1	Lepidoptera	Lepidoptera
27	**Q**	L	T	F	T	S	S	**W**	G		Manse-AKH [9]	6	Lepidoptera	Lepidoptera Hymenoptera
28	**Q**	L	T	F	T	S	S	**W**	G	G	Lacol-AKH [24]	2	Lepidoptera	Lepidoptera
29	**Q**	L	T	Y	S	P	G	**W**			Novel [7]	1	Hemiptera	Hemiptera
30	**Q**	V	N	F	S	P	G	**W**			Manto-CC [4]	5	Ephemeroptera Phasmida	Mantophasmatodea Hemiptera Blattodea
31	**Q**	V	N	F	S	P	G	**W**	G	Q	Litfo-AKH [30]	1	Lepidoptera	Chilopoda, Lithobiomorpha
32	**Q**	V	N	F	S	P	G	**W**	G	T	Bladi-HrTH [29]	3	Blattodea	Blattodea
33	**Q**	V	N	F	S	P	N	**W**			Peram-CAH-I [13]	15	Coleoptera	Blattodea Coleoptera Hemiptera Myriapoda
34	**Q**	V	N	F	S	P	N	**W**	G	G	Olisa-AKH [11]	2	Hemiptera	Embioptera Hemiptera
35	**Q**	V	N	F	S	P	S	**W**			Anaim-AKH [11]	10	Thysanoptera Phasmida Collembola, Symphypleona	Odonata Hemiptera
36	**Q**	V	N	F	S	P	T	**W**			Galyu-AKH [11]	1	Hemiptera	Grylloblattodea
37	**Q**	V	N	F	S	P	Y	**W**	N	Q	Letin-AKH long [11]	1	Hemiptera	Hemiptera

Table 1. Continued.

#	1	2	3	4	5	6	7	8	9	10	AKH	n	Predicted in order	Known in order
38	**Q**	V	N	F	S	T	G	**W**			Grybi-AKH [11]	5	Hymenoptera	Orthoptera Hemiptera Dermaptera Neuroptera Coleoptera Hymenoptera
39	**Q**	V	N	F	T	P	G	**W**			Psein-AKH [11]	3	Odonata	Odonata Mantophasmatodea Hemiptera
40	**Q**	V	N	F	T	P	N	**W**			Emppe-AKH [24]	4	Psocodea Blattodea	Mantodea Hemiptera Coleoptera
41	**Q**	V	N	F	T	P	S	**W**			Libau-AKH [31]	2	Mantophasmatodea Odonata	Odonata
42	**Q**	V	N	F	T	P	T	**W**	G	Q	Acypi-AKH [11]	2	Hemiptera	Hemiptera

All mature AKHs have pQ in position 1, and they are C-terminally amidated. Some AAs were marked in grey for improved visualisation. References indicate reviews or latest mention. Positions 1 and 8 (Q and W) as fixed AA are marked in bold.

sawfly (pQLNFSTGWGGam, entry 70 [15]). The expression of the same octapeptide extended by residues GK (entry 71) has been suggested for different Coleopteran species before by other authors [32]. Decapeptides 12 and 13 share seven AA core residues except for Lys6 with Eriss-CC (pQLTFSAGWam, Diptera, entry 144 [10]), Dipma-AKH (pQLTFSDWGNam, Hemiptera, entry 145 [11]), Glomo-AKH (pQLTFSPGWam, Diptera, Hemiptera, entry 155 [4]) and Antya-AKH (pQLTFSPGWGQam, Lepidoptera, entry 157 [9]) and were predicted in Coleoptera (entries 146–148).

Sequences 14 and 15 (QLTFSRDWAG, QLTFSRDWSA, entries 169–173) reside in the alphabetical list between octapeptides Volpe-CC (pQLTFSPYWam, entry 168, Diptera [4]) and Piebr-AKH (pQLTFSSGWam, entry 174, Lepidoptera, Hemiptera [9]), but they are decapeptides of unique composition suggested for Lepidoptera.

Number 16 and 17 (QLTFTPGWGF, QLTFTPGWGS, entries 202, 207) are derived from Tabat-AKH (pQLTFTPGWam, entry 201, Diptera [24]) and strongly resemble Bommo-AKH-II (pQLTFTPGWGQam, entry 206, Lepidoptera [9]) and Tabat-HoTH (pQLTFTPGWGYam, entry 208, Diptera [24]) except for AA residue 10. They were predicted in a dipteran and a hemipteran species.

For two dipteran species, the presence of sequence 18 (QLTFTPQW, entries 218, 219) was proposed. It differs in position 6 from Peram-CAH-II (pQLTFTPNWam, entry 210, found in several orders [24]) and Aedae-AKH (pQLTFTPSWam, entry 220, Diptera [10]).

Decapeptide 19 (QLTFTSSWGT, entry 243, Lepidoptera) was detected for the Eastern spruce budworm and it is related to Hipes-AKH-I (pQLTFTSSWam, entry 230), Manse-AKH (pQLTFTSSWGam, entry 231), Lacol-AKH (pQLTFTSSWGGam, entry 232), Hipes-AKH-II (pQLTFTSTWam, entry 244) and Hipes-AKH-III (pQLTFTSTWGam, entry 245), all validated in Lepidoptera [9].

Octapeptide 20 (QLTFTTGW, entry 246), predicted for Diptera, differs in positions 6 and 7 from the 8-AA core of the three Hipes-AKHs. Sequence 21 (QLTYSTDW, entry 253, Hymenoptera) has its closest equivalents in Nicve-AKH (pQLTYSTGWam, entry 254, Coleoptera [12]) and a novel AKH predicted by others in Hemiptera (QLTYSTNW, entry 255 [11]).

Peptide 22 (QVNFSPAW, entries 256, 257) was suggested for two springtail species and differs only in Ala7 from Manto-CC (pQVNFSPGWam, entry 258 [4]), which was found in species of several orders. Number 23 stands for Manto-CC extended by Gly in position 9 (entry 261, Blattodea) and is related to decapeptides such as Litfo-AKH (pQVNFSPGWGQam, entry 267 [30]) found in a centipede and predicted for a saddleback caterpillar (entry 268) as well as Bladi-HrTH (pQVNFSPGWGTam, entry 269 [29]) known from Blattodea (predicted for three more Blattodea species, entries 270–272).

Sequence 24 represents a rather unusual AA composition with the large Trp residue in position 7 (QVNFTPWW, entry 338, Orthoptera). A peptide with the also large Tyr7 has already been predicted for Hemiptera (entry 339) [11]. Other AKHs with differences in position 7 include Emppe-AKH (pQVNFTPNWam, entry 326, several orders [24]) and Libau-AKH (pQVNFTPSWam, entry 332, Odonata [31]). Related decapeptide Rommi-CC (pQVNFTPNWGTam, entry 331 [12]) was found in Orthoptera and Acypi-AKH (pQVNFTPTWGQam, entry 335 [11]) in Hemiptera.

Sequences 25–27 (QVTFSKGWGP, QVTFSNGWKP, QVTFSNGWNQ, entries 346–353, Hymenoptera) bear resemblance to the ACPs (see above), but they are different in positions 6 and 7. One of the proproteins of a wasp was classified as “hypertrehalosaemic prohormone-like” in the DB (entry 347). The last sequence 28 (QVTFTRDWA, entry 442, Neuroptera), predicted for an antlion, brackets the ACPs with Thr in position 5, but it has been flagged for its unusual SP processing site ATT (see above).

Table 2. Predicted novel sequences (#) for putative AKHs in the context of known AKHs summarized from Supplementary Tables 1,2 and sorted alphabetically.

Sequence	#	AKH	Order	Species	Common name
QFNFSAGW		Cirba-AKH	Coleoptera	*Circellium bacchus*	Flightless dung beetle
QINYS*P*GW	1		Ephemeroptera	*Cloeon dipterum*	Mayfly
QINYSTGW		Harax-AKH	Coleoptera	*Harmonia axyridis*	Lady beetle
QITFSRDW*KN*	2		Hemiptera	*Macrosteles quadrilineatus*	Aster leafhopper
QITFSRDW*MG*	3		Lepidoptera	*Eumeta japonica*	Bagworm moth
QITFSRDW*NA*	4		Orthoptera	*Gryllus longicercus*	Cricket
QITFSRDWSG		Bommo-AKH-III	Lepidoptera	*Bombyx mori*	Domestic silk moth
QITFSRDW*TC*	5		Lepidoptera	*Ostrinia furnacalis*	Grass moth
QITFSRDW*TG*	6		Lepidoptera	*Achroia grisella*	Lesser wax moth
QITFST*D*W*AQ*	7		Hymenoptera	*Cyphomyrmex costatus*	Fungus-growing ant
QITFSTGW		Cimle-AKH	Hemiptera	*Cimex lectularius*	Common bed bug
QLNFSTDW		Trica-AKH	Coleoptera	*Tribolium castaneum*	Red flour beetle
QLNFSTDW*GQ*	8		Hymenoptera	*Wasmannia auropunctata*	Little fire ant
QLNFST*E*W	9		Hymenoptera	*Dinoponera quadriceps*	Ant
QLNFSTGW		Schgr-AKH-II	Orthoptera Coleoptera Hemiptera Hymenoptera	*Schistocerca gregaria*	Desert locust
QLNFSTGWGG		Tenar-HrTH	Hymenoptera Blattodea	*Tenthredo arcuata*	Sawfly
QLNFSTGW*GQ*	10		Hymenoptera	*Acromyrmex echinatior*	New World ant
QLNFSTGW*SQ*	11		Hymenoptera	*Pogonomyrmex barbatus*	Harvester ant
QLTFSAGW		Eriss-CC	Diptera	*Eristalis ssp*	Hoverfly
QLTFSDGWGN		Dipma-AKH	Hemiptera	*Dipetalogaster maxima*	Kissing bug
QLTFS*K*GW*KI*	12		Coleoptera	*Diabrotica virgifera virgifera*	Western corn rootworm
QLTFS*K*GW*KV*	13		Coleoptera	*Diorhabda carinulata*	Northern tamarisk beetle
QLTFSPGW		Glomo-AKH	Diptera Hemiptera	*Glossina morsitans*	Tsetse fly
QLTFSPGWGQ		Antya-AKH	Lepidoptera	*Antherae yamamai*	Japanese silk moth
QLTFSPYW		Volpe-CC	Diptera	*Volucella pellucens*	Pellucid fly
QLTFS*RD*W*AG*	14		Lepidoptera	*Parnassius mnemosyne*	Swallowtail butterfly
QLTFS*RD*W*SA*	15		Lepidoptera	*Iphiclides podalirius*	Scarce swallowtail
QLTFSSGW		Piebr-AKH	Lepidoptera Hemiptera	*Pieris brassicae*	Cabbage butterfly
QLTFTPGW		Tabat-AKH	Diptera	*Tabanus atratus*	Horsefly
QLTFTPGWG*F*	16		Diptera	*Uranotaenia lowii*	Mosquito
QLTFTPGWGQ		Bommo-AKH-II	Lepidoptera	*Bombyx mori*	Domestic silk moth
QLTFTPGWG*S*	17		Hemiptera	*Rhynocoris fuscipes*	True bug
QLTFTPGWGY		Tabat-HoTH	Diptera	*Tabanus bovinus*	Pale giant horsefly
QLTFTPNW		Peram-CAH-II	Blattodea Coleoptera Lepidoptera Hemiptera Orthoptera	*Periplaneta americana*	American cockroach
QLTFTP*Q*W	18		Diptera	*Bradysia coprophila*	Fungus gnat
QLTFTPSW		Aedae-AKH	Diptera Megaloptera	*Aedes aegypti*	Mosquito
QLTFTSSW		Hipes-AKH I	Lepidoptera	*Hippotion eson*	Sphinx moth
QLTFTSSWG		Manse-AKH	Lepidoptera Hymenoptera	*Manduca sexta*	Tobacco hawk moth
QLTFTSSWGG		Lacol-AKH	Lepidoptera	*Lacanobia oleracea*	Moth
QLTFTS*S*WG*T*	19		Lepidoptera	*Choristoneura fumiferana*	Eastern spruce budworm moth
QLTFTSTW		Hipes-AKH II	Lepidoptera	*Hippotion eson*	Sphinx moth
QLTFTSTWG		Hipes-AKH III	Lepidoptera	*Hippotion eson*	Sphinx moth
QLTFT*TG*W	20		Diptera	*Topomyia yanbarensis*	Mosquito
QLTYST*D*W	21		Hymenoptera	*Harpegnathos saltator*	Ant
QLTYSTGW		Nicve-AKH	Coleoptera	*Nicrophorus vespilloides*	Burying beetle
QVNFSP*A*W	22		Collembola, Entomobryomorpha	*Folsomia candida*	Springtail
QVNFSPGW		Manto-CC	Mantophasmatodea Hemiptera Blattodea	*Mantophasma kudubergense*	Heel-walker

Table 2. Predicted novel sequences (#) for putative AKHs in the context of known AKHs summarized from Supplementary Tables 1,2 and sorted alphabetically.

Sequence	#	AKH	Order	Species	Common name
QVNFSPGW*G*	23		Blattodea	*Lucihormetica verrucosa*	Warty glowspot cockroach
QVNFSPGWGQ		Litfo-AKH	Chilopoda, Lithobiomorpha	*Lithobius forficatus*	Garden centipede
QVNFSPGWGT		Bladi-HrTH	Blattodea	*Blaberus discoidalis*	False death's head cockroach
QVNFTPNW		Emppe-AKH	Mantodea Hemiptera Coleoptera	*Empusa pennata*	Conehead mantis
QVNFTPNWGT		Rommi-CC	Orthoptera Coleoptera	*Romalea microptera*	Grasshopper
QVNFTPSW		Libau-AKH	Odonata	*Libellula auripennis*	Golden-winged skimmer
QVNFTPTWGQ		Acypi-AKH	Hemiptera	*Acyrthosiphon pisum*	Pea aphid
QVNFTP*W*W	24		Orthoptera	*Schistocerca cancellata*	Locust
QVTF*SKG*W*GP*	25		Hymenoptera	*Aphidius gifuensis*	Wasp
QVTF*SNG*W*KP*	26		Hymenoptera	*Cephus cinctus*	Wheat stem sawfly
QVTF*SNG*W*NQ*	27		Hymenoptera	*Pseudomyrmex gracilis*	Graceful twig ant
QVTFSRDWNA		Rhopr-ACP	Hemiptera	*Rhodnius prolixus*	Kissing bug
QVTFSTGW		Novel AKH	Coleoptera	*Anoplophora glabripennis*	Asian long-orned beetle
QVTF*TRD*W*A*	28		Neuroptera	*Myrmeleon formicarius*	Antlion
QYNFSTGW		Oniay-CC-I	Coleoptera	*Onitis aygulus*	Two-toned beetle

Sequence-wise, the canon of known AKHs is bracketed by Cirba-AKH and Oniay-CC. For details and species, see **Supplementary Tables 1,2**. Note that all mature AKHs have pQ in AA position 1 and they are C-terminally amidated. Changes in AA composition to the nearest known AKH in the list are in italic and coloured in red. The special case of Rhopr-ACP (see text) is marked in grey.

3.5 Screening in Arthropoda

Supplementary Table 1 also includes the few AKHs, which were detected and validated in other classes of the Arthropoda than insects such as Strma-AKH [33] and Litfo-AKH [30] (Chilopoda, entries 7, 267), Panbo-RPCH [29] (Malacostraca, entry 50), Manto-CC [30] (Diplopoda and Symphyla, entries 259, 260), Argsi-RPCH [7] (Maxillopoda, entry 319), and Dappu-RPCH (Branchiopoda, entry 320). The BLAST search in Arthropoda added 58 entries (**Supplementary Table 4**, 867 entries), of which 33 suggest the presence of Panbo-RPCH (entry 91) in different Malacostraca species and 8 show sequence data for the presence of Dappu-RPCH [7] (entry 671) in Daphnia species. One match from Diplopoda has the 8-AA core of Manto-CC (entry 571) extended by residues GG and differs from Litfo-AKH (entry 582) only by Gly instead of Gln in position 10. Entries for two Pycnogonida and one Ostracoda match and share the basic octapeptide with Grybi-AKH [11] (entry 659). Entry 639 indicates Anaim-AKH [11] in Collembola. Three more entries have the same sequence part QVNFSP, but vary in positions 7, 9 and 10 (Collembola, Chilopoda).

Two sequences (QITFSPKWTP, "hypothetical protein" in Arachnida obtained by soft tick genome assembly, entry 23; QVTFSRGWEA, "uncharacterised protein" found by genome assembly for the sea spider, Pycnogonida, entry 862) seem to be unique results for putative AKHs.

Four entries were extracted by the search, which refer to Maxillopoda decapeptides derived from the octapeptide Manse-AKH (entry 500). However, three of them concern the same species (*Pollicipes pollicipes*), and at least for one of these entries the SP cleavage site does not conform to the rules (see above) and was flagged. There are two more questionable hits (entries 87, Chilopoda; 566, Arachnida) with atypical AA residues at both the SP cleavage site and in the 8-AA core sequence. Entry 565 shows a rather unusual Thr in position 2 and was thus marked (Chilopoda).

3.6 Limitations

The study was successful in that it extracted 28 new potential AKHs in insects and 42 known AKHs in 162 species for which they have not yet been investigated. The sequences were assembled in a searchable archive (**Supplementary Tables 1–5**). The collection allows grouping the entries, e.g., for AKHs in certain taxonomic orders or families. The workflow required considerable manual data curation, not only to remove duplicates, but also to eliminate false hits not meeting search requirements, bringing down the initial output in the high tens of thousands to valuable information of close to 10.000 entries. A second round of editing was subsequently concerned with plausibility considerations regarding the SP sequence and the knowledge about ACPs. Third, the hits were compared to the so far investigated species in order to compile a list of predicted (not yet validated) AKHs. It is a limitation of such global screenings that specific details or publications of individual hits may have been missed, but several cross-checks indicated that their number should be marginal.

Due to the design of the search based on similarity, it cannot be excluded that potential sequence matches may have been missed. However, during several early attempts to develop an appropriate workflow, it was observed that changing input parameters collected rather more of the same hits than finding new ones, indicating that not

much more was there to be found. Moreover, the search was based on the known AKHs and the sequence rules derived from them. While the algorithm still reports sequences slightly outside these requirements (see discussion about outliers above), this experimental design may also be a cause of missed entries, but again, early trials instilled confidence in the process.

Incidentally, for the sake of simplicity, one might be tempted to extend the search to the entire NCBI DB in a global and completely unbiased approach. Besides computing and analysis times, there are several other reasons why this is neither useful nor practical. From a biological point of view, the AKH signalling system is well defined and described in insects, and, even though the invertebrate AKH receptors show homology to the vertebrate gonadotropin-releasing hormone receptors, hormone structures differ [1,2]. Thus, even if hits conforming to the search requirements were reported for other animals, they would be coincidental without biological meaning and simply clutter the results. In fact, lots of bacteria seem to have sequence stretches which fit the search parameters. Moreover, the BLAST algorithm itself does not perform in the same quality for a huge global DB as for smaller specific DBs due to, among programming parameters, the number of testable sequences (search space) and output restrictions.

Furthermore, while it is technically possible to perform phylogenetic analysis with short peptides such as AKHs [34], it is not as informative and accurate as when using preprohormone sequences for the purpose. This was, however, beyond the scope and goal of the present work. Also, the DB screening did not focus on the biology and history of individual AKHs, but rather on their sequence composition.

4. Conclusion

This investigation used peptide AA sequence comparison and alignment to extract putative hormone sequences from public NCBI DBs to visualize the known and validated insect AKHs within the ensemble of potential (predicted from genomics/transcriptomics information) and hypothetical (calculated sequence variations) peptides of this characteristic structure with the aim of building a framework to contextualize new samples. The result is a searchable and sortable collection as a reference for the analytical process of identifying hormones in new species. Sequence similarity search predicted 28 novel AKHs in insects and the presence of 42 known AKHs in 162 species, which have not yet been investigated experimentally for such peptide hormones. In addition, the screening in Arthropoda provided 58 more entries, most of which were for known AKHs, but also two for potentially novel peptides.

The screening process involved the examination of the preprohormone processing sites based on the published knowledge for SP cleavage and dibasic processing. In the majority of the known AKHs, AA residues GKR follow the sequence stretch of the AKH in the prohormone; the tag GRR was rarely observed. SP processing sites for known AKHs are typically of the form A/C/G/S/T/V × A/C/G/S and agree with the (–3,–1)-rule [18]. Predicted peptides indicate that Leu/Ile may be allowed at the –3-position and Thr at the –1-position, but this needs to be experimentally confirmed.

As a result of their structural similarity, this work also detected ACPs, which represent a different signalling system from the AKHs; they were not the focus of the present study. Still, eight novel ACP-like sequences differing in positions 9 and 10 were extracted and may be of interest to researchers in that field.

While the topic-guided DB search for predicted AKHs has become standard in current AKH research [13,14], the presented unbiased screening provides a snapshot of the entire current, publicly available through NCBI, AKH hormone landscape. With ongoing rapid sequencing endeavours, it will be outdated quickly and should be repeated from time to time, possibly including SP prediction with SignalP 6.0 software [35]. However, AKH research has advanced considerably during the last years so that already this peptide collection, including mass information, provides an extensive reference tool to which new discoveries can be easily added.

Abbreviations

AA, amino acid; ACP, AKH/corazonin-related peptide; AKH, adipokinetic hormone; CC, corpora cardiaca; DB, database; NCBI, National Center for Biotechnology Information; SP, signal peptide.

Availability of Data and Materials

Data are provided in the Supplement. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

SK performed the BLAST searches, data analysis including Visual Basic programming. She has prepared, read and approved the final manuscript, and agrees to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The author declares no conflicts of interest. Given her role as an Editorial Board member, Simone König had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Graham Pawelec.

Declaration of AI and AI-Assisted Technologies in the Writing Process

Visual Basic code was developed with assistance from the large language model Gemini. Alignment analysis was conducted using NCBI web tools; while these services may integrate algorithmic or machine learning optimization, the specific underlying computational architecture remains undisclosed by the platform. After using this tool, the author reviewed and edited the result as needed and took full responsibility for the content of the publication.

Supplementary Material

An Excel file provides database entries obtained by NCBI Blast search. Sequences are sorted alphabetically. If available, the N-terminal amino acid residues of the signal peptide are shown as well as the dibasic processing site. All sequence information can be accessed via https://www.ncbi.nlm.nih.gov with one exception (*access via Uniprot). Animal class is insects with the exceptions shown. A selection of AA residues is colored for better visualization. Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL55493.

References

[1] Marco HG, Gäde G. Adipokinetic hormone: A hormone for all seasons? Advances in invertebrate (NEURO) endocrinology (pp. 129–175). Apple Academic Press: Burlington ON, Canada. 2020.

[2] Marco HG, Glendinning S, Ventura T, Gäde G. The gonadotropin-releasing hormone (GnRH) superfamily across Pancrustacea/Tetraconata: A role in metabolism? Molecular and Cellular Endocrinology. 2024; 590: 112238. https://doi.org/10.1016/j.mce.2024.112238

[3] Stork NE. How Many Species of Insects and Other Terrestrial Arthropods Are There on Earth? Annual Review of Entomology. 2018; 63: 31–45. https://doi.org/10.1146/annurev-ento-020117-043348

[4] König S, Marco HG, Gäde G. The proline effect and the tryptophan immonium ion assist in de novo sequencing of adipokinetic hormones. Scientific Reports. 2023; 13: 10894. https://doi.org/10.1038/s41598-023-38056-2

[5] Eipper BA, Park LP, Dickerson IM, Keutmann HT, Thiele EA, Rodriguez H, et al. Structure of the precursor to an enzyme mediating COOH-terminal amidation in peptide biosynthesis. Molecular Endocrinology (Baltimore, Md.). 1987; 1: 777–790. https://doi.org/10.1210/mend-1-11-777

[6] Eisenacher M, de Braaf J, König S. Mass analysis peptide sequence prediction (MAPSP). Bioinformatics (Oxford, England). 2006; 22: 1002–1003. https://doi.org/10.1093/bioinformatics/btl052

[7] Gäde G, Šimek P, Marco HG. Novel members of the adipokinetic hormone family in beetles of the superfamily Scarabaeoidea. Amino Acids. 2016; 48: 2785–2798. https://doi.org/10.1007/s00726-016-2314-0

[8] Gäde G, Šimek P, Marco HG. Structural diversity of adipokinetic hormones in the hyperdiverse coleopteran Cucujiformia. Archives of Insect Biochemistry and Physiology. 2019; 102: e21611. https://doi.org/10.1002/arch.21611

[9] Marco HG, Šimek P, Gäde G. Unique Members of the Adipokinetic Hormone Family in Butterflies and Moths (Insecta, Lepidoptera). Frontiers in Physiology. 2020; 11: 614552. https://doi.org/10.3389/fphys.2020.614552

[10] Gäde G, Šimek P, Marco HG. The Adipokinetic Peptides in Diptera: Structure, Function, and Evolutionary Trends. Frontiers in Endocrinology. 2020; 11: 153. https://doi.org/10.3389/fendo.2020.00153

[11] Gäde G, Marco HG. The Adipokinetic Peptides of Hemiptera: Structure, Function, and Evolutionary Trends. Frontiers in Insect Science. 2022; 2: 891615. https://doi.org/10.3389/finsc.2022.891615

[12] Gäde G, König S, Marco HG. Structural Diversity of Adipokinetic Hormones in the Coleopteran Suborder Polyphaga (Excluding Cucujiformia). Archives of Insect Biochemistry and Physiology. 2025; 118: e70049. https://doi.org/10.1002/arch.70049

[13] Marco HG, König S, Gäde G. Predicted novel hypertrehalosaemic peptides of cockroaches are verified by mass spectrometry. Amino Acids. 2023; 55: 1641–1654. https://doi.org/10.1007/s00726-023-03337-7

[14] Marco HG, König S, Gäde G. Mass Spectrometric Proof of Predicted Peptides: Novel Adipokinetic Hormones in Insects. Molecules (Basel, Switzerland). 2022; 27: 6469. https://doi.org/10.3390/molecules27196469

[15] Jiang S, Marco HG, Scheich N, He S, Wang Z, Gäde G, et al. Comparative analysis of adipokinetic hormones and their receptors in Blattodea reveals novel patterns of gene evolution. Insect Molecular Biology. 2023; 32: 615–633. https://doi.org/10.1111/imb.12861

[16] O'Shea M, Rayne RC. Adipokinetic hormones: cell and molecular biology. Experientia. 1992; 48: 430–438. https://doi.org/10.1007/BF01928161

[17] Hook V, Funkelstein L, Lu D, Bark S, Wegrzyn J, Hwang SR. Proteases for processing proneuropeptides into peptide neurotransmitters and hormones. Annual Review of Pharmacology and Toxicology. 2008; 48: 393–423. https://doi.org/10.1146/annurev.pharmtox.48.113006.094812

[18] von Heijne G. A new method for predicting signal sequence cleavage sites. Nucleic Acids Research. 1986; 14: 4683–4690. https://doi.org/10.1093/nar/14.11.4683

[19] Choo KH, Ranganathan S. Flanking signal and mature peptide residues influence signal peptide cleavage. BMC Bioinformatics. 2008; 9: S15. https://doi.org/10.1186/1471-2105-9-S12-S15

[20] Nielsen H, Tsirigos KD, Brunak S, von Heijne G. A Brief History of Protein Sorting Prediction. The Protein Journal. 2019; 38: 200–216. https://doi.org/10.1007/s10930-019-09838-3

[21] Seidah NG, Prat A. The biology and therapeutic targeting of the proprotein convertases. Nature Reviews. Drug Discovery. 2012; 11: 367–383. https://doi.org/10.1038/nrd3699

[22] Gäde G, König S, Kulkarni SS, Payne RJ, Marco HG. Threonine sulfation: a rare post translational modification in insect adipokinetic hormones. Scientific Reports. 2026; 16: 22190. https://doi.org/10.1038/s41598-026-50205-x

[23] König S, Albers C, Gäde G. Mass spectral signature for insect adipokinetic hormones. Rapid Communications in Mass Spec-

trometry: RCM. 2005; 19: 3021–3024. https://doi.org/10.1002/rcm.2167

[24] Marco HG, König S, Gäde G. The adipokinetic hormones of stored product pest insects: can this help to combat these insects? Journal of Stored Products Research. 2026; 117: 102998. https://doi.org/10.1016/j.jspr.2026.102998

[25] Hansen KK, Stafflinger E, Schneider M, Hauser F, Cazzamali G, Williamson M, et al. Discovery of a novel insect neuropeptide signaling system closely related to the insect adipokinetic hormone and corazonin hormonal systems. The Journal of Biological Chemistry. 2010; 285: 10736–10747. https://doi.org/10.1074/jbc.M109.045369

[26] Zandawala M, Haddad AS, Hamoudi Z, Orchard I. Identification and characterization of the adipokinetic hormone/corazonin-related peptide signaling system in Rhodnius prolixus. The FEBS Journal. 2015; 282: 3603–3617. https://doi.org/10.1111/febs.13366

[27] Siegert KJ. Locust corpora cardiaca contain an inactive adipokinetic hormone. FEBS Letters. 1999; 447: 237–240. https://doi.org/10.1016/s0014-5793(99)00299-9

[28] Zhu C, Huang H, Hua R, Li G, Yang D, Luo J, et al. Molecular and functional characterization of adipokinetic hormone receptor and its peptide ligands in Bombyx mori. FEBS Letters. 2009; 583: 1463–1468. https://doi.org/10.1016/j.febslet.2009.03.060

[29] König S, Marco HG, Gäde G. Oxidation Products of Tryptophan and Proline in Adipokinetic Hormones-Artifacts or Post-Translational Modifications? Life (Basel, Switzerland). 2023; 13: 2315. https://doi.org/10.3390/life13122315

[30] Derst C, Dircksen H, Meusemann K, Zhou X, Liu S, Predel R. Evolution of neuropeptides in non-pterygote hexapods. BMC Evolutionary Biology. 2016; 16: 51. https://doi.org/10.1186/s12862-016-0621-4

[31] Gäde G. Peptides of the adipokinetic hormone/red pigment-concentrating hormone family: a new take on biodiversity. Annals of the New York Academy of Sciences. 2009; 1163: 125–136. https://doi.org/10.1111/j.1749-6632.2008.03625.x

[32] Veenstra JA. Coleoptera genome and transcriptome sequences reveal numerous differences in neuropeptide signaling between species. PeerJ. 2019; 7: e7144. https://doi.org/10.7717/peerj.7144

[33] Chipman AD, Ferrier DEK, Brena C, Qu J, Hughes DST, Schröder R, et al. The first myriapod genome sequence reveals conservative arthropod gene content and genome organisation in the centipede Strigamia maritima. PLoS Biology. 2014; 12: e1002005. https://doi.org/10.1371/journal.pbio.1002005

[34] Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, et al. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. Nucleic Acids Research. 2019; 47: W260–W265. https://doi.org/10.1093/nar/gkz303

[35] Teufel F, Almagro Armenteros JJ, Johansen AR, Gíslason MH, Pihl SI, Tsirigos KD, et al. SignalP 6.0 predicts all five types of signal peptides using protein language models. Nature Biotechnology. 2022; 40: 1023–1025. https://doi.org/10.1038/s41587-021-01156-3