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Identification and characterization of antimicrobial peptides from *Feijoa sellowiana* transcriptome

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Abstract

Antimicrobial peptides (AMPs) are small polypeptides present in a wide diversity of phylogenetically distant organisms, including plants. They are part of the innate immune response, allowing rapid action at a lower energetic cost than the adaptive immune system. Various applications of these peptides as anti-infective agents have been demonstrated. Despite their significant potential, the initiatives for bioprospecting of AMPs from the native flora of Uruguay are still incipient. The aim of this study was to identify and characterize genes encoding six antimicrobial peptides families, comprising: defensins, snakins, thionins, hevein-like, lipid transfer proteins (LTPs), and cyclotides in the native fruit species *Feijoa sellowiana* (Myrtaceae), commonly known as *guayabo del país* or *feijoa*. The search was carried out using BLAST, based on a *de novo* transcriptome of leaf and flower, utilizing reference sequences. The relationships between *F. sellowiana* and reference protein sequences were characterized through multiple alignments and phylogenetic analysis. The exon-intron structure and isoforms of *F. sellowiana* AMPs were analyzed by PCR amplification in genomic DNA. This study identified 23 defensins, 49 snakins, 7 thionins, 12 hevein-like and 87 LTPs; no evidence of cyclotide transcripts was found. The expected exon-intron structure was confirmed for five sequences, belonging to defensins, snakins, and hevein-like families.

Keywords: AMPs, guayabo del país, gene validation, *de novo* transcriptome, defensins

Identificación y caracterización de péptidos antimicrobianos en el transcriptoma de *Feijoa sellowiana*

Resumen

Los péptidos antimicrobianos (AMPs) son polipéptidos pequeños, presentes en una gran diversidad de organismos alejados filogenéticamente, incluidas las plantas. Forman parte de la respuesta inmune innata, permitiendo una acción rápida a menor costo energético que el sistema inmune adaptativo. Se han demostrado diversas aplicaciones de estos péptidos como agentes antiinfecciosos. A pesar de su importante potencial, la bioprospección de AMPs en la flora nativa de Uruguay es aún incipiente. El objetivo de este estudio fue identificar y caracterizar genes que codifican seis fami-

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lias de péptidos antimicrobianos que comprenden: defensinas, esnaquinas, tioninas, *hevein-like*, proteínas de transferencia de lípidos (*lipid transfer proteins* [LTPs]) y ciclótidos, en la especie frutal nativa *Feijoa sellowiana* (Myrtaceae), comúnmente llamada guayabo del país. La búsqueda se realizó mediante BLAST, a partir de un transcriptoma *de novo* de hoja y flor y utilizando secuencias de referencia. Las relaciones entre *F. sellowiana* y secuencias de proteínas de referencia se caracterizaron mediante alineamientos múltiples y análisis filogenético. La estructura exón-intrón y las isoformas de los AMP de *F. sellowiana* se analizaron mediante amplificación por PCR en ADN genómico. Este estudio identificó 23 defensinas, 49 esnaquinas, 7 tioninas, 12 *hevein-like* y 87 LTPs, no encontrándose evidencia de transcriptos de ciclótidos. La estructura exón-intrón esperada se confirmó para cinco secuencias, pertenecientes a familias de defensinas, esnaquinas y *hevein-like*.

Palabras clave: AMPs, guayabo del país, validación génica, transcriptoma *de novo*, defensinas

Identificação e caracterização de peptídeos antimicrobianos no Transcriptoma de *Feijoa sellowiana*

Resumo

Os peptídeos antimicrobianos (AMPs) são pequenos polipeptídios presentes em uma grande diversidade de organismos filogeneticamente distantes, incluindo plantas. Eles fazem parte da resposta imunológica inata, permitindo uma ação rápida com um custo energético menor do que o sistema imunológico adaptativo. Diversas aplicações desses peptídeos como agentes anti-infecciosos foram demonstradas. Apesar de seu significativo potencial, a bioprospecção de AMPs na flora nativa do Uruguai ainda é incipiente. O objetivo deste estudo foi identificar e caracterizar genes que codificam seis famílias de peptídeos antimicrobianos compreendendo: defensinas, snakins, tioninas, hevein-like, proteínas de transferência de lipídios (LTPs) e ciclotídeos, na espécie frutífera nativa *Feijoa sellowiana* (Myrtaceae), comumente conhecida como goiabeira-serrana. A busca foi realizada usando BLAST, baseada em um transcriptoma *de novo* de folha e flor, utilizando seqüências de referência. As relações entre *F. sellowiana* e sequências de proteínas de referência foram caracterizadas por meio de alinhamentos múltiplos e análise filogenética. A estrutura exon-íntron e isoformas de AMPs de *F. sellowiana* foram analisadas por amplificação por PCR em DNA genômico. Este estudo identificou 23 defensinas, 49 snakins, 7 tioninas, 12 *hevein-like*, 87 LTPs; nenhuma evidência de transcrições de ciclotídeo foi encontrada. A estrutura exon-íntron esperada foi confirmada para cinco seqüências, pertencentes às famílias defensinas, esnaquinas e *hevein-like*.

Palavras-chave: AMPs, goiabeira-serrana, validação gênica, transcriptômica *de novo*, defensinas

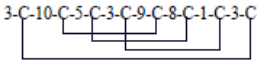
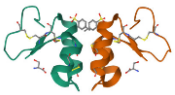
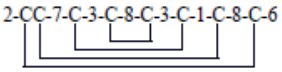
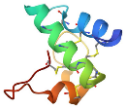
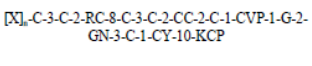
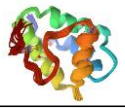
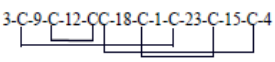
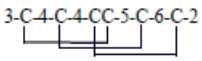
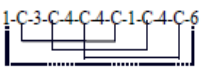
1. Introduction

Antimicrobial peptides (AMPs) are ancient defense weapons with a potential role in the evolutionary success of multicellular organisms. As part of the innate immune response, these proteins allow a rapid response at a lower energy cost compared to the adaptive immune system of higher vertebrates[(1)]. They are present in both plants and animals, playing an important role in forming defense barriers either before or after infection, against a wide range of target organisms, including bacteria, fungi, viruses, and protozoa[(1)]. AMPs consist of polypeptides of less than 200 amino acids (often fewer than 50 amino acids), and unlike secondary metabolites, they are products of unique genes[(2)(3)].

Despite a great diversity of AMPs has been reported, certain features are typically shared among the AMP families, such as their small size, general positive charge, amphipathic stereogeometry, tolerance to acidic and organic solvents, thermal stability, and broad biological activity[(4)(5)]. In addition, they commonly present an even number of cysteine residues that participate in intramolecular disulfide bridges, conferring structural and thermodynamic stability[(2)].

In plants, AMPs are frequently secreted to the extracellular space, and are found constitutively in storage organs (e.g. seeds) and in the outer layers of cells in generative tissues (reproductive organs, fruits, and flowers). In vegetative tissues, they are also produced induced by infections or wounds[3][6]. Several plant-derived AMP families have been identified based on conserved and specific arrangements of cysteine residues, which leads to similar folding patterns and structures, even in the absence of a high degree of sequence similarity or identity. The six major AMP families comprise defensins, thionins, lipid transfer proteins (LTP), cyclotides, hevein-like proteins, and snakins[3][7] (**Table 1**).

Table 1. Description of 3D structure and cysteine arrangements of representative antimicrobial peptides (AMP) from six major AMP families

Family	Name	3D Structure[a]	Cysteine arrangement
Defensins	Rs-AFP2		
Thionins	Beta-hordothionin		
Snakins	StSN1		
LTPs	Lc-LTP2		
Hevein-like	Hevein		
Cyclotides	Kalata B1		

Modified from Lay and Anderson[2] and Benko-Iseppon and others[3].

[a] 3D structures were taken from Protein Data Bank (PDB)[8]. β strands are schematically represented as a ribbon, which usually ends in an arrow to indicate the direction of the chain, α helix are represented as a spiral ribbon.

Various applications of AMPs as anti-infectious agents have been demonstrated[1]. In the agronomy field, AMPs are considered promising compounds for the prevention and treatment of commercially important crop diseases and for post-harvest preservation[9][10], as well as for enhancing tolerance to abiotic stress[11]. Additionally, recent interest in developing new AMP-based human therapeutic agents from AMPs is driven by the growing problem of resistance to conventional antibiotics and the need for new antibiotics[1].

So far, commercial crops have been the primary target in the search for AMPs. In contrast, the bioprospection of AMPs in wild species is still in its early stages. The greater biodiversity present in wild plants increases the relevance of bioprospecting AMP in this category of species[12]. Actually, several AMPs with confirmed antimi-

crobial activity have been recently identified by bioprospecting several wild plant species native to Uruguay, including *Peltophorum dubium* (Spreng.) Taub. (ibirapitá), *Erythrina crista-galli* L. (ceibo), and *Maytenus ilicifolia* Mart. ex Reissek (congorosa)[13][14][15][16].

Feijoa sellowiana (O. Berg) O. Berg., commonly known as *feijoa* or *guayabo del país*, is a woody, fleshy-fruit species belonging to the Myrteae tribe of Myrtaceae. It is an open-pollinated, diploid (2n=22) species, native to Uruguay and Brazil, which is highly appreciated for its edible fruits and as ornamental[17]. Currently, there is a growing interest in bioprospecting biologically active compounds in *F. sellowiana*, as a result of the findings of antioxidant, anti-inflammatory, antimicrobial, anticancer, and antidiabetic activities identified from *F. sellowiana* leaf, flower, and fruit extracts[18]. However, until now, AMPs have not yet been studied in this species. Recently, a *de novo* transcriptome of leaf and floral tissues of *F. sellowiana* has been reported[19], enabling an *in-silico* search of AMP. The objective of this work was to identify and characterize genes encoding antimicrobial peptides of major AMP families: defensin, snakin, thionin, hevein-like, LTP, and cyclotide in *F. sellowiana de novo* transcriptome.

2. Materials and Methods

2.1 AMP Prediction in *Feijoa sellowiana* Transcriptome

The analysis started with the results from the *de novo* assembly of the transcriptome[19], and transcripts were examined for putative sequences encoding AMP-type proteins, specifically from the defensin, snakin, thionin, hevein-like, cyclotide, and LTP families. The BioEdit program[20] was used to create a local database with the transcriptome pool. The local BLAST tool (tblastN) within the same program was used, with query sequences reported from AMP proteins in different species. The reference AMP sequences reported in other plant species were obtained from UniProt[21], Phytozome[22], and NCBI[23] (**Table S1**). Hits with an E-value less than 0.01 were selected, and at least three cysteines matching the typical motifs of each peptide were required. The motifs of the families were based on those described by Slavokhotova and others[24].

2.2 Alignments and Neighbor-Joining Trees

Multiple alignments of the predicted primary *Feijoa sellowiana* proteins and reference sequences used as queries were performed using ClustalW within BioEdit[20]. To infer phylogenetic relationships, these alignments were then used to construct cladograms using the MEGA program, employing the Neighbor-Joining method[25].

2.3 Exon-Intron Structure of AMP Genes

Five defensin, one hevein-like and three snakin/GASA putative genes were PCR amplified from *Feijoa sellowiana* genomic DNA. Primers were designed from transcript contigs with the aid of Primer3 Plus program to target the region corresponding to the mature peptide and/or in 5' and 3' UTRs (**Table S2**). Genomic DNA extraction from *F. sellowiana* leaves was performed using the method of Doyle[26]. PCR amplification was performed in a 20 µl reaction containing: 1X DreamTaq buffer (Thermo) with 2 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 µM of each primer, 50 ng template DNA, 1 ng BSA, and 1 U DreamTaq DNA polymerase (Thermo). The PCR program was as follows: 94 °C for 3 min; followed by 35 cycles of 94 °C for 30 sec, 52-58 °C for 40 sec and 72 °C for 40 sec. The amplified fragments were visualized by agarose gel electrophoresis. The sequencing of genomic amplified AMP genes was performed at Macrogen Inc. (Seoul, Korea). To analyse exon-intron structure and detect possible transcript isoforms, PCR products and their corresponding transcript contigs were aligned using Clustal W and Pairwise Alignment tools in BioEdit.

3. Results

3.1 Sequence Identification

As a result of the BLAST process and manual curation, 177 sequences with similarity to AMPs were identified (**Table 2**, **Table S3** a-e). Most of the sequences had at least two isoforms. The sequences were named using the prefix As (*Acca sellowiana,* used as a synonym of *Feijoa sellowiana*), followed by the family name (Df for defensins, Sn for snakins, Tn for thionins, Hv for heveins, and LTP for LTPs), and a consecutive number. These sequences comprise both complete (Signal Peptide presence and cysteine domain complete) and nearly complete AMP coding sequences (CDS) that may lack the Signal peptide and/or showed incomplete cysteine motifs (**Table 2**, **Table S3**).

Table 2. Number of AMP sequences identified in the *Feijoa sellowiana* transcriptome, classified by family, based on a homology-guided search

Family	N° of sequences identified
Defensins	23
Snakin/GASA	49
Thionins	7
Hevein-like	11
Cyclotides	0
LTPs	87
TOTAL	177

3.2 Multiple Alignments and Cladograms

For defensins, a multiple alignment of the of 23 *Feijoa sellowiana* predicted peptides (AsDf) was performed against six (**Table S1**) of 15 *Eucalyptus grandis* defensins, which this study previously annotated on *Eucalyptus grandis v2.0* complete genome assembly. Based on the phylogenetic analysis, the 23 AsDf and six *E. grandis* defensins were clustered in four groups with at least one *E. grandis* sequence in each one (**Figure S1**). To further describe these groups, **Figure 1** showed the multiple alignment of these sequences. The two typical distinctive defensin motifs: the Csαβ motif ($CX_nCX_3CX_nCX_nCXC$) (where C=Cys, X=any amino acid, and n indicates a non-conserved number of amino acids (aa)), and the γ-core motif ($GXCX_{3-9}C$) were present in all AsDf. However, in some cases, these motifs were incomplete. Also, 12 of 23 exhibited signal peptide (**Table S3** a). A total of 11 AsDf (1, 2a, 2b, 3-7, 10-11, 13) that showed both signal peptide as well as complete cysteine motifs were considered complete. Based on the number of aminoacids placed between cysteine 1 (Cys 1) and 2 (Cys 2), four groups were identified (**Figure 1**, **Figure S1**). Accordingly, for Group 1-4, Cys1 and Cys2 were separated by 10, 5, 6 and 8 aa, respectively. In addition, Group 2 showed an additional Cys at the amino terminal end. Furthermore, different patterns of conserved residues were found among the four groups.

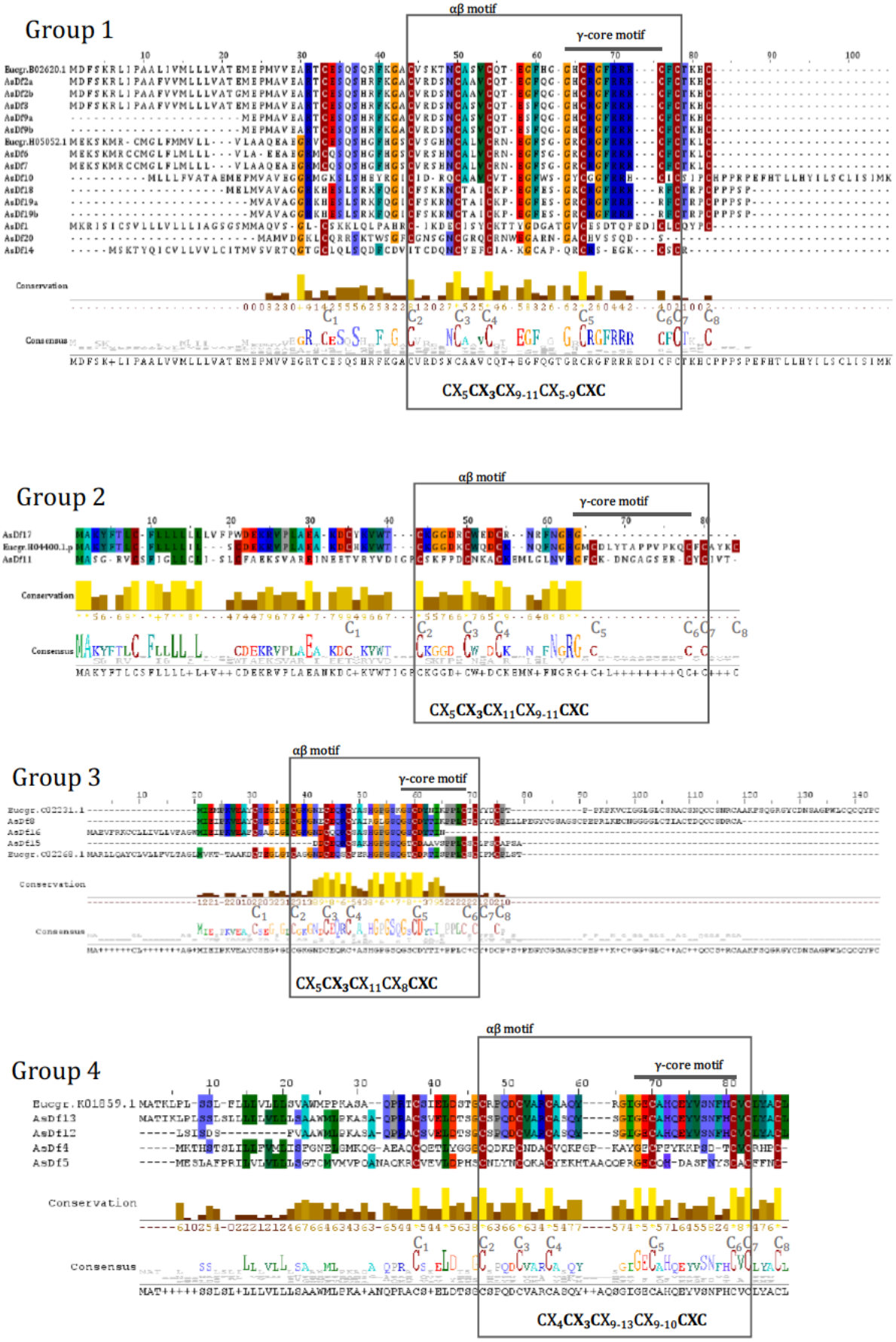


Figure 1. Alignment of *Feijoa sellowiana* defensin sequences identified with *Eucalyptus grandis* reference sequences

The sequences were grouped according to their similarity identifying four groups. In all four cases, the αβ motifs (CXnCXXXCXnCXnCXC) and γ-core (GXCX3-9C) are presented. Threshold (%) for Identity/Similarity shading was 60%.

In the case of snakins, the alignment of 49 AsSn sequences was performed against the reference snakin sequences StSN1, StSN2, and StSN3 from potato (*Solanum tuberosum*), each belonging to a distinct subfamily, namely 1, 2, and 3, respectively[27]. The phylogenetic analysis revealed a clustering into three main groups that coincided with subfamilies 1, 2, and 3. The smallest group (subfamily 1) comprised 12 *Feijoa sellowiana* snakins, while the largest group (subfamily 2) comprised 22 (**Figure 2**, **Figure S2**).

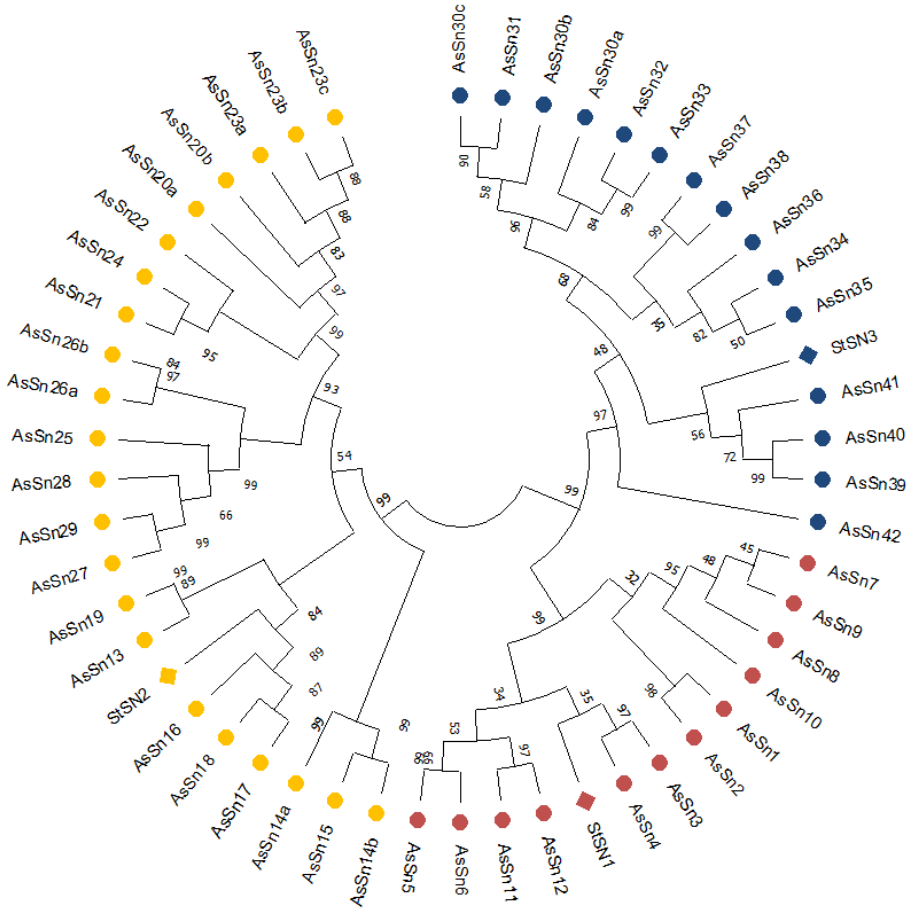


Figure 2. Neighbor Joining tree (cladogram) of 49 putative Snakin/GASA peptides (AsSn) found in the *Feijoa sellowiana* transcriptome and potato snakins StSn1, StSn2 and StSn3 as references

Diamonds represent potato snakins, and circles represent *F. sellowiana* proteins. Subfamily 1 is shown in red; subfamily 2 in yellow, and subfamily 3 in blue. Node values indicate bootstrap values (5000 replicates)

For thionins, the seven candidate sequences were aligned against two reference sequences from *Eucalyptus grandis*, as well as revised sequences from *Triticum aestivum* (wheat), *Hordeum vulgare* (barley), *Arabidopsis thaliana*, and *Viscum album*. A pattern of Cys was shared between all *Feijoa sellowiana* and *E. grandis* sequences but not with *T. aestivum, H. vulgare, A. thaliana*, and *V. album*, as shown in **Figure S3**. Regarding the NJ tree, three groups were observed, two of which were solely composed of *F. sellowiana* and *E. grandis* sequences, while the third one (Group 3) comprised revised sequences from the remaining species (**Figure 3**, **Figure S3**).

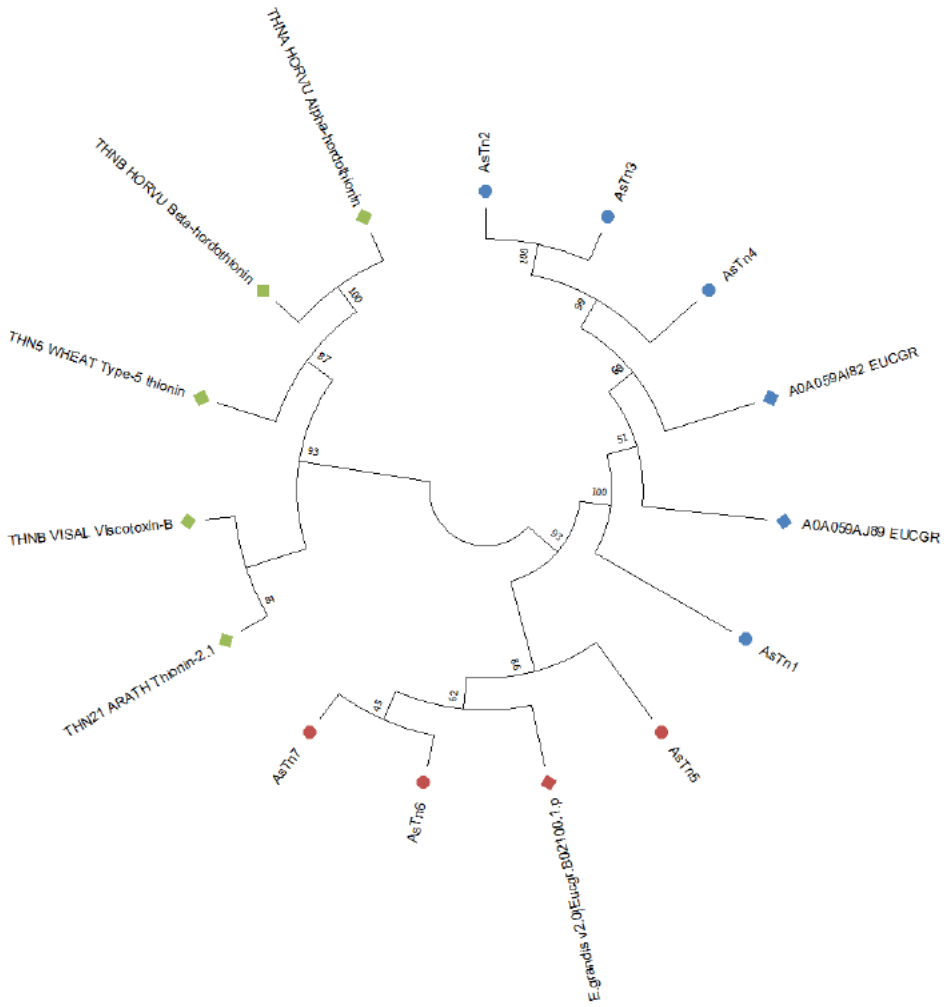


Figure 3. Neighbor Joining tree of genetic distance (cladogram) of seven putative thionin peptides found in the *Feijoa sellowiana* transcriptome and reference sequences from *Eucalyptus grandis*, *Triticum aestivum* (wheat), *Hordeum vulgare* (barley), *Arabidopsis thaliana*, and *Viscum album*

Diamonds represent the reference thionins, and circles represent *F. sellowiana* proteins. Group 1 is shown in blue, Group 2 in red, and Group 3 in green. Node values indicate bootstrap values (5000 replicates).

For hevein-like proteins, the hevein motif of 11 *Feijoa sellowiana* peptides were aligned against reference hevein from *Hevea brasiliensis*, along with revised sequences from *Stellaria media, Triticum kiharae*. In addition, six *Eucalyptus grandis* sequences that showed similarity with putative hevein sequences from *F. sellowiana* were also included (**Figure 4**). Two groups were detected with *F. sellowiana* putative heveins. Group 1 included seven *F. sellowiana* heveins as well as reference proteins from *H. brasiliensis, S. media, T. kiharae*, and one protein from *E. grandis*; while Group 2 exclusively comprised four *F. sellowiana* and five reference *E. grandis* heveins.

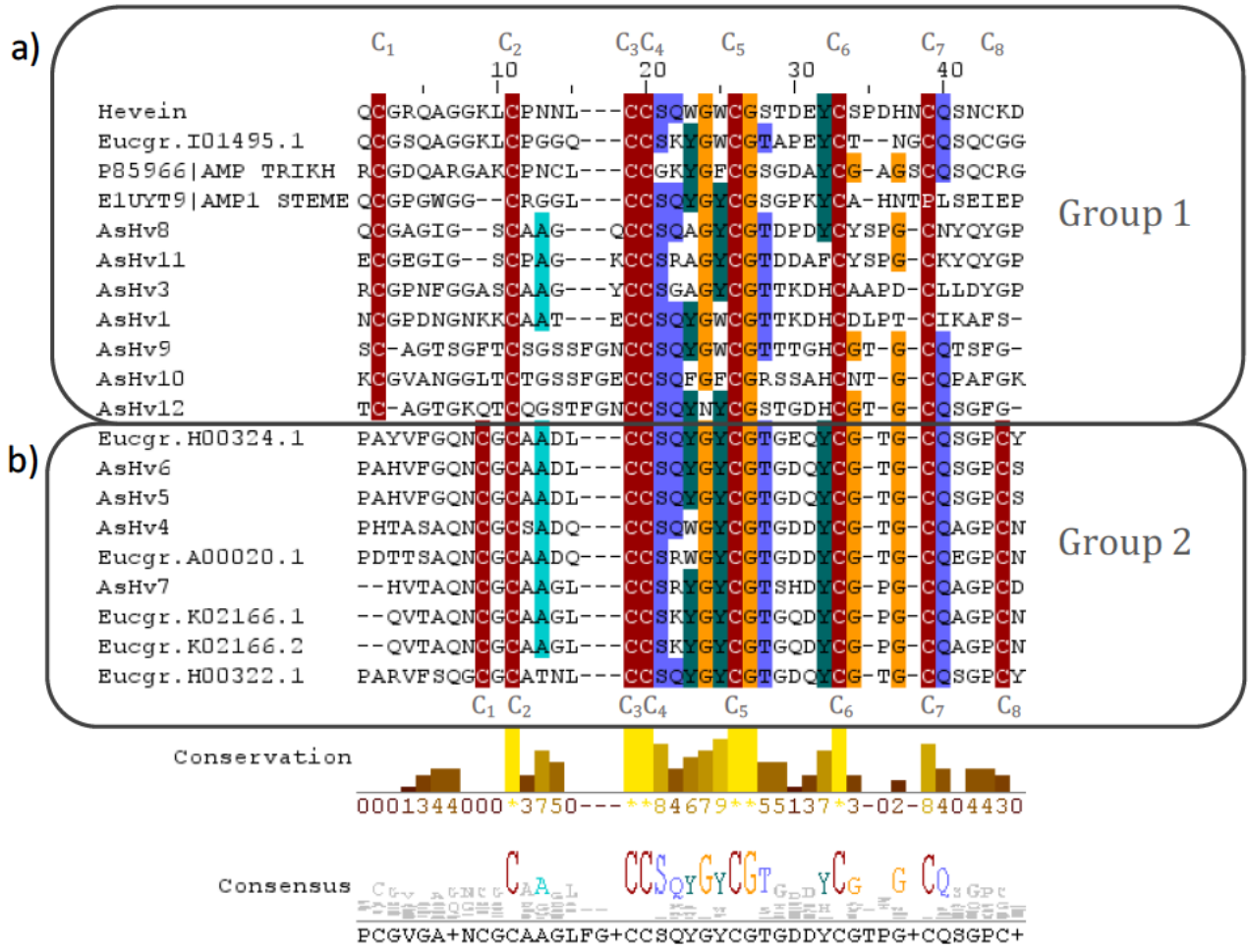


Figure 4. Alignment of the hevein motif from 11 hevein-like sequences of *Feijoa sellowiana* with reference sequences *from Eucalyptus grandis, Stellaria media, Triticum kiharae*, and the hevein from *Hevea brasiliensis:* a) Group 1, b) Group 2

A total of 87 *Feijoa sellowiana* putative LTPs were found. The multiple alignment (data not shown) and cladogram (**Figure 5**) using revised reference sequences from *Arabidopsis thaliana*, *Lens culinaris, Vigna radiata* and *Prunus armeniaca* revealed three main groups (**Figure 5**). Of the four reference sequences belonging to the LTP1 subfamily, three were placed in Group 2 (blue) (A0AT29, Q42589, and Q39176), while one was placed in Group 1 (green) (A0A1S3TFR4). A reference sequence from the LTP2 subfamily (P82353) was placed in Group 3 (red). The AsLTP proteins were distributed in all three groups. Thirty-two and 41 AsLTPs are assigned to Groups 1 and 2, respectively, which may correspond to the LTP1 subfamily, while remaining 14 AsLTPs were found in the LTP2 group (Group 3).

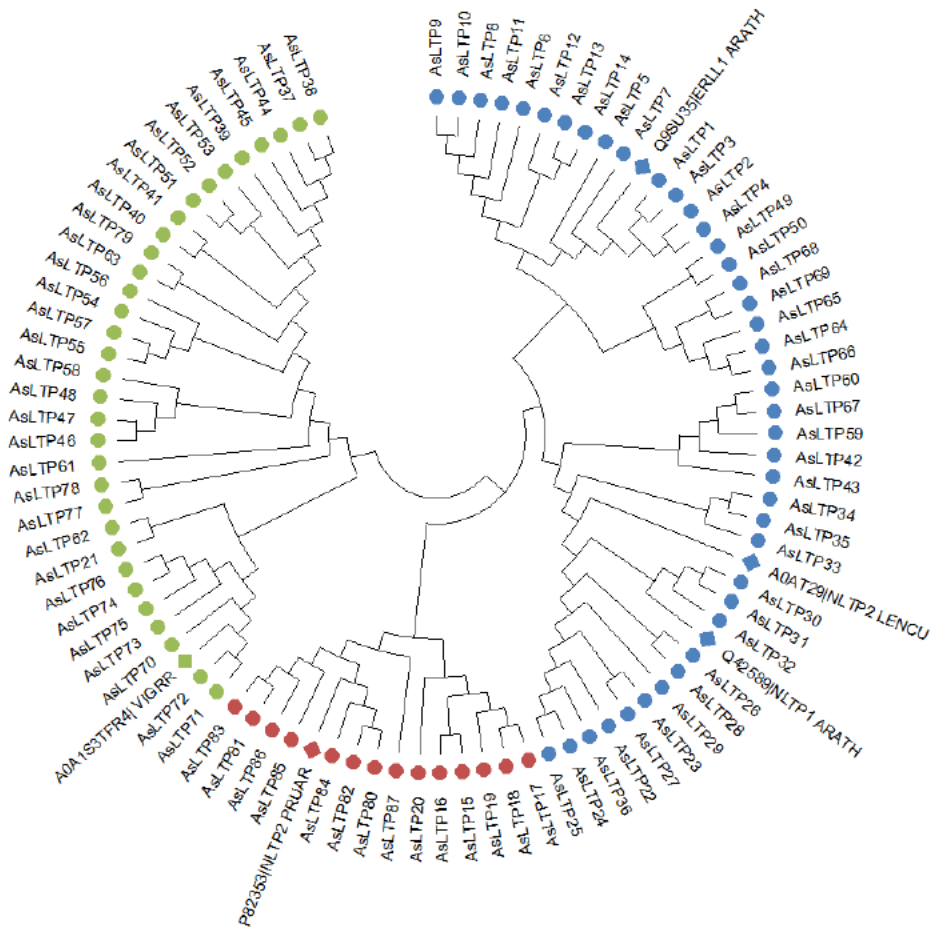


Figure 5. Neighbor Joining tree (cladogram) of 87 putative LTP peptides found in the *Feijoa sellowiana* transcriptome and reference sequences from *Arabidopsis thaliana*, *Lens culinaris, Vigna radiata*, and *Prunus armeniaca*

Diamonds represent the reference LTPs, and circles represent *F. sellowiana* proteins. Group 1 is shown in green, Group 2 in blue, and Group 3 in red.

3.3 Exon-Intron Structure

Nine *Feijoa sellowiana* candidate AMP transcript sequences comprising five complete defensins, one complete hevein-like, and three snakins were selected for characterizing the exon-intron structure. Out of the 9 amplified products from genomic DNA, only five sequences (AsDf1, AsDf5, AsDf7, AsSn1 and AsHv1) showed adequate quality. For AsDf1, the alignment of the transcript and genomic sequences revealed a single gap corresponding to an intron of 143 bp long (**Figure 6** a). An additional nucleotide (A) was added at nucleotide 407 due to the poor quality of the sequence, which caused the reading frame to shift from +2 to +3 at this position. Similarly, an exon-intron-exon arrangement was also found for AsDf5 and AsDf7 (**Figure 6** b-c) for which intron lengths were 205 and 90 bp, respectively. Interestingly, the alignment of transcript AsDf6 and genomic AsDf7 revealed 100% identity (data not shown). As transcript sequences for AsDf6 and AsDf7 showed 100% identity at both exon regions, AsDf6 may represent an AsDf7 isoform, exhibiting intron retention. For AsSn1 and AsHv1, an exon-intron-exon structure was also observed (**Figure 6** d-e) with intron lengths of 108 and 90 bp, respectively.

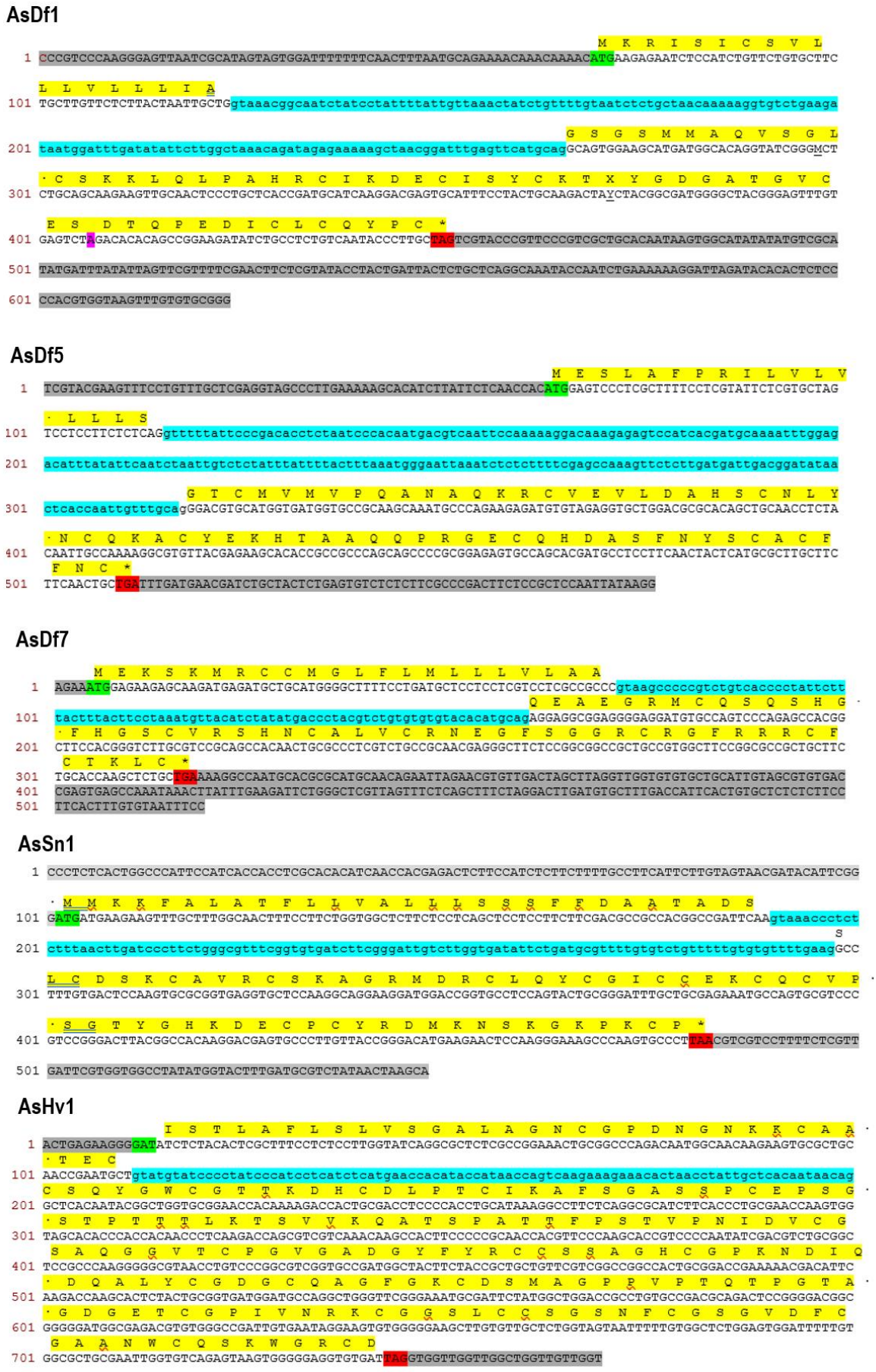


Figure 6. Gene structure and protein sequences of *Feijoa sellowiana* AsDf1, AsDf5, AsDf7, AsSn1 and AsHv1

The 3' and 5' UTR (untranslated regions) are highlighted in grey, the start codon in light green, the stop codon in red, the intron in light blue, and the protein-coding sequence in yellow. AsDf1: The added nucleotide is marked in fuchsia. Two underlined nucleotides indicated lack of consensus between the forward and reverse sequences.

4. Discussion

AMPs have proven to be highly promising molecules for anti-infective use in various fields[1]. While an ample number of plant-derived AMP have been identified from several plant species, very little has been done on the identification of AMP from Myrtaceae. This study identified, for the first time, a large number of five AMP families in a *Feijoa sellowiana de novo* transcriptome assembly based on homology with reference AMPs, and manual curation. The outcomes of this study expand the repertoire of functional AMPs in leaf and flower tissues as well as the origin of plant-derived AMP.

Plant defensins generally consist of a signal peptide at the amino-terminal end, and a basic, cysteine-rich mature peptide, with typical αβ and γ-core motifs, of 45 to 54 aa long[3]. *In silico* search for plant defensins based on transcriptome data has been reported. Rodríguez Decuadro[13] reported 14 defensins from *Peltophorum dubium* and 12 defensins from *Maytenus ilicifolia*. Slavokhotova and others[28] identified 16 transcripts encoding defensins in healthy (non-infected) seeds of *Stellaria media*. This study identified 23 *Feijoa sellowiana* defensins that clustered in four groups and revealed, for the first time, their relationships with those from *Eucalyptus*. Although four groups have not been previously reported in the literature, each of them presented at least one reference sequence from *Eucalyptus grandis*.

Defensin genes exhibit a typical exon-intron-exon structure[29]. In this study, a clear exon-intron-exon structure was found for AsDf1 and AsDf5. As for AsDf6, the alignment between the amplified genomic sequence and the corresponding contig (transcript) did not reveal the presence of any intron. However, when the alignment was performed with AsDf7 transcript (a putative isoform of AsDf6), an exon-intron-exon structure was observed. These results may indicate that AsDf6 and AsDf7 are true isoforms, or that the Df6 may represent an unprocessed transcript that erroneously retained an intron.

Snakins are encoded by snakin/GASA genes and are characterized by having a GASA domain of approximately 60 aa, with 12 cysteine residues in highly conserved positions, that may be involved in the formation of up to six disulfide bonds[11]. In this study, both alignment and clustering analyses supported that snakins, including those from *Feijoa sellowiana*, were clustered into three groups, which corresponded to the subfamilies described in potato based on SNSt1, SNSt2, and SNSt3, as described by Segura and others[30], Berrocal-Lobo and others[27], and Nahirñak and others[11]. Other studies have also divided the snakin/GASA proteins into three highly conserve subfamilies across different species[31][32][33]. The phylogenetic analysis of the *F. sellowiana* Snakin/GASA predicted proteins further supported the classification of snakins in three subfamilies

A total of 49 snakin/GASA genes were detected, all of which, except for three, were denoted as isoforms according to Trinity assembler. In the work by Rodríguez Decuadro[13][16] putative snakin transcripts were predicted in *Peltophorum dubium* and 15 transcripts in *Maytenus ilicifolia*, while Li and others[31] reported 18 putatitve snakin transcripts in *Nicotiana tabacum*. Shang and others[32] predicted 27, 30, and 9 snakin-GASA genes in the mangroves, *Avicennia marina*, *Kandelia obovata*, and *Aegiceras corniculatum*, respectively. Sun and others[33] identified 15 snakin/GASA genes in the genome of *Brassica rapa* L. ssp. pekinensis. These numbers are somewhat lower than those found in this work, suggesting that the higher number could be due to isoforms. However, in the *Eucalyptus grandis* genome, there are three proteins annotated as "snakin" and 38 annotated with the GASA motif[34]. The similar number to that in *E. grandis* might indicate that the number of snakins in the Myrtaceae family could be higher.

The exon-intron structure of snakin genes varied among subfamilies. For subfamily 1, snakin genes consist of two exons and one intron, while for subfamilies 2 and 3, the total number of introns varied between two and three[35]. The genomic sequence of snakin AsSn1 exhibited an exon-intron-exon structure that corresponded with the expected structure of genes from the subfamily 1. This result was consistent with the placement of AsSn1 within subfamiliy 1, based on phylogenetic results (**Figure 2**). Further work on the analysis of AsSn13

(subfamily 2) and AsSn42 (subfamily 3) gene structures would allow us to verify whether the expected exon-intron structure for each of these two subfamilies had also been retained in these *Feijoa sellowiana* genes.

Hevein is a small chitin-binding protein domain of 43 aa long, derived from a prohevein, which is encoded by a hevein precursor gene originally described for the rubber tree (*Hevea brasiliensis*)[36]. Eleven hevein-like sequences were found in *Feijoa sellowiana* transcriptome, with two of them as potential isoforms. These numbers were similar to those reported in other species using the same methodology. In *Peltophorum dubium*, eight hevein-like transcripts were found, while in *Maytenus ilicifolia*, ten transcripts were identified[13]. In *Leymus arenarius*, two hevein-like sequences were identified[24], and in healthy *Stellaria media* seeds[28], 14 hevein-like sequences were found. In *Eucalyptus*, five hevein transcripts are annotated in the Phytozome database[34]. Most of hevein-like peptides possess eight cysteine residues forming four disulfide bridges. Out of the 12 heveins found in *F. sellowiana*, none of those placed in Group 1 showed a complete set of 8 cysteines, whereas all from Group 2 did. Regarding the AsHv1 gene sequence (hevein), the presence of an intron was identified. The literature regarding the structure of hevein genes is scarce. Van Damme and others[37] reported two introns for a hevein-like gene expressed in the fruit of *Sambucus nigra*. However, a recent study on the hevein gene family in *Hevea brasiliensis* reported that the presence of one intron was conserved in the four members of this family[38].

Thionins are basic peptides reported in various plant tissues such as seeds, stems and roots, and are mainly located in the intracellular space (although they can also be found extra-cellularly)[39]. In this work, seven putative transcripts were obtained, two of which appeared as isoforms. The numbers reported for *Peltophorum dubium* and *Maytenus ilicifolia* are 0 and 1, respectively[13]. In *Leymus arenarius*, 15 thionins were reported[24], and in the transcriptome of *Stellaria media*, three were described[28]. In *Eucalyptus grandis*, seven thionins are also annotated[32], suggesting that this may be a number associated with phylogenetic proximity. The pre-pro-thionin protein consists of a signal or leader peptide of 25 amino acids and a pro-peptide of 60 amino acids (which includes the mature peptide and an acidic C-terminal tail, which neutralizes the mature thionin)[40]. The reference thionin sequences used in this work (except for those from eucalyptus) formed an exclusive group; consistently, marked differences in the cysteine patterns between these peptides and those found in *Feijoa sellowiana* were observed in the alignments. This result suggested that the *F. sellowiana* thionins differed from those used as reference organisms. However, the *Eucalyptus* thionins showed greater homology with those from *F. sellowiana*, and in the cladogram, they clustered together into two groups. Notably, the *Arabidopsis thaliana* thionin-like sequences reported in the work of Almaghrabi and others[41], in particular from the CRP2310 and CRP2360 families, showed the same cysteine pattern as that of putative thionins of *F. sellowiana.*

All LTPs shared a conserved pattern of eight cysteines at specific positions. They have been subdivided into two subfamilies, according to their molecular masses. As such, peptides from LPT1 and LPT2 show ca. 10 and 7 kDa, respectively[42]. A total of 87 AsLTPs were found in this work, but isoforms were not characterized due to the large quantity. Rodríguez Decuadro[13] reported 28 putative LTPs for *Peltophorum dubium* and 32 for *Maytenus ilicifolia*, while Slavokhotova and others[28] reported 51 LTPs, and 31 for healthy *Stellaria media* seeds[26]. However, in *Eucalyptus grandis*, 91 proteins are annotated as "lipid transfer proteins"[32], suggesting, as with thionins, that this may be associated with phylogenetic proximity. In this study, *Feijoa sellowiana* LTP peptides were clustered with both LPT1 and LPT2 references peptides, although most of them corresponded to LPT1 variants. Rodríguez Decuadro[16] found that LTP1 sequences from *P. dubium* were divided into three groups, where reference LTP1 from *Vigna radiata* A0A1S3TFR4 was positioned in one of them, separated from the other LTP1 reference sequences. Similarly, in this work, the *V. radiata* sequence was placed in Group 1, along with other sequences from *F. sellowiana*, separated from the other LTP1 reference sequences placed in Group 3. According to Santos-Silva and others[43], physicochemical variables should be included with both phenetic and functional characteristics to generate a more appropriate functional classification system for this group of peptides, as the classification system into subfamilies 1 and 2 appeared to be artefacts.

Cyclotides are short cyclic peptides that have a head-to-tail cyclized backbone and three conserved disulfide-bonds in a knotted arrangement[44]. So far, they have been mainly found in species of the Rubiaceae, Cucurbitaceae, Violaceae, Solanaceae, Poaceae, and Fabaceae families[45]. No reports of cyclotides have been found in Myrtaceae and no protein annotated as "cyclotide" is found in the *Eucalyptus grandis* genome stored at Phytozome either. Accordingly, in this study, no evidence of cyclotide transcripts was found in *Feijoa sellowiana* transcriptome. Recent studies aimed to identify cyclotides in *Peltophorum dubium* (Fabaceae) and *Maytenus ilicifolia* (Celastraceae) genomes led also to negative results[13]. In order to confirm whether cyclotides are truly absent in these genomes, it might be worth considering more sensitive, alternative search approaches to Blast.

5. Conclusions

Using a bioinformatics approach, 177 AMPs including 23 defensins, 49 snakins, 7 thionins, 11 hevein-like, and 87 LTPs were identified in a *de novo* transcriptome of *Feijoa sellowiana*. No sequence with homology to cyclotides was found. Notably, several *F. sellowiana* defensin, thionin and hevein-like AMPs were found as likely members of novel groups within each of these AMP families. Conversely, for snakins and LTPs, the proteins from *F. sellowiana* were similar to those already characterized in other species. For five of *F. sellowiana* AMP, both the expected size and gene structure were validated. Altogether, this study identified, for the first time in Myrtaceae, a diverse AMP repertoire, and provided evidence of novel AMP diversity that warrant additional exploration. Further studies on tissue and temporal-expression patterns of the *F. sellowiana* AMP genes, as well as on the antimicrobial activity of corresponding AMP proteins will be shortly initiated.

Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author Contribution Statement

	F Rossi	C Pritsch	S Rodriguez Decuadro
Conceptualization		■	■
Funding acquisition		■	
Investigation	■		
Methodology			■
Supervision			■
Writing – original draft	■		
Writing – review and editing		■	■

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Supplementary Material

Table S1. Query sequences used for BLAST searches by AMP family. The accession number corresponds to the Uniprot, Phytozome, or NCBI databases, depending on the protein

AMP	Species	Accession Number	Sequence query
Snakin	*Solanum tuberosum*	Q948Z4 · SNAK1_SOLTU	MKLFLLTLLLVTLVITPSLIQTTMAGSSFCDSKCKLRCSKAGLADRCLKYCGICCEECKCVPSGTYGNK HECPCYRDKKNSKGKSKCP
		Q93X17 · SNAK2_SOLTU	MAISKALFASLLLSLLLLEQVQSIQTDQVTSNAISEAAYSYKKIDCGGACAARCRLSSRPRLCNRACGT CCARCNCVPPGTSGNTETCPCYASLTTHGNKRKCP
		M1BA38 · M1BA38_SOLTU	MAKSGYNASFLLLLSMFLILLTFSNVVEGYNKLRPRDCKPKCTYRCSATSHKKPCMFFCQKCCATCL CVPKGVYGNKQSCPCYNNWKTQEGKPKCP
Hevein	*Hevea brasiliensis*	|P02877|HEVE_HEVB	EQCGRQAGGKLCPNNLCCSQWGWCGSTDEYCSPDHNCQSNCKD
	Amaranthus caudatus	|P27275|AMP_AMACA	MVNMKCVALIVIVMMAFMMVDPSMGVGECVRGRCPSGMCCSQFGYCGKGPKYCGRASTTVDHQA DVAATKTAKNPTDAKLAGAGSP
	Stellaria media	|E1UYT9|AMP1_STEME	MLNMKSFALVMLFATLVGVTIASGPNGQCGPGWGGCRGGLCCSQYGYCGSGPKYCAHNTPLSEIEP TDAGRCSGRGTCSGGRCCSKYGYCGTGPAYCGLGMCQGSCLPDMPNHPAQIQARTEAAQAEAQAEA YNQANEAAQVEAYYQATQAQTQAQPQVEPAVTKAP
	Triticum kiharae	|P85966|AMP_TRIKH	AQRCGDQARGAKCPNCLCCGKYGFCGSGDAYCGAGSCQSQCRGCR
LTP	*Arabidopsis thaliana*	|Q9SU35|ERLL1_ARATH **LTP1**	MASKNSASLALFFALNILFFTLTVATNCNCKPSPKPKPVPSPKPKPVQCPPPPRPSVPSPNPRPVTPP RTPGSSGNSCPIDALKLGVCANVLSSLLNIQLGQPSSQQCCSLIQGLVDVDAAICLCTALRANVLGINL NVPISLSVLLNVCNRKLPSGFQCA
		|Q42589|NLTP1_ARATH **LTP1**	MAGVMKLACLLLACMIVAGPITSNAALSCGSVNSNLAACIGYVLQGGVIPPACCSGVKNLNSIAKTTP DRQQACNCIQGAARALGSGLNAGRAAGIPKACGVNIPYKISTSTNCKTVR
	Lens culinaris	|A0AT29|NLTP2_LENCU **LTP1**	MARGMKLACVVLVICMVVIAPMAEGAISCGAVTSDLSPCLTYLTGGPGPSPQCCGGVKKLLAAANTT PDRQAACNCLKSAAGSITKLNTNNAAALPGKCGVNIPYKISTTTNCNTVKF
	Vigna radiata var. radiata	|A0A1S3TFR4|_VIGRR **LTP1**	MEGVVKFACLVGFVVLVSVAKVDSAGECGKSTTPDNEAIKLAPCASAAQDENASVSQSCCAQVKKIG QNPSCLCAVLLSNTAKMAGVNPQIAVTIPKRCNLANRPVGYKCGPYTLP
	Prunus armeniaca	|P82353|NLTP2_PRUAR **LTP2**	VTCSPVQLSPCLGPINSGAPSPTTCCQKLREQRPCLCGYLKNPSLRQYVNSPNARKLASNCGVPVPQC
Thionins	*Hordeum vulgare*	P01545 · THNA_HORVU	MVCLLILGLVLEQVQVEGKSCCRSTLGRNCYNLCRVRGAQKLCAGVCRCKLTSSGKCPTGFPKLALVS NSDEPDTVKYCNLGCRASMCDYMVNAAADDEEMKLYLENCGDACVNFCNGDAGLTSLTA
		P21742 · THNB_HORVU	MGSKGLKGVMVCLLILGLVLEHVQVEGKSCCRSTLGRNCYNLCRVRGAQKLCANACRCKLTSGLKCP SSFPKLALVSNSDEPDTIDYCNLGCRASMCDYMVNAAADDEEMKLYVEHCSDACVNFCNGDVGLTS LTA
	Triticum aestivum	Q05806 · THN5_WHEAT	MGGGQKGLESAIVCLLVLGLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVFQCADFCACRLPAGL ASVRSSDEPNAIEYCSLGCRSSVCDNMINTADNSTEEMKLYVKRCGVACDSFCKGDTLLASLDD
	Eucalyptus grandis	A0A059AJ89_EUCGR	MDKVKSVLVVCLVLGLFLGQSRADFQDCYVGCFVACVVTGDNVVKCSLKCLKDCIGLPSHGLTDAEY FCKLGCASSLCINLSSKDDPGEKRVAHCVNSCSKTCANHA
		A0A059AI82_EUCGR	MGKVKSVFMVCLVLGLFLGQSGANFQDCYPACFILCAITPGRTLFSCSVECLKDCIIPPSDSLSLRDTM YFCKLGCASSLCTNLSTKDNPREKRVAGCVNSCSKTCTDHV
Defensins	*Eucalyptus grandis*	Eucgr.B02620.1.p	MDFSKRLIPAALIVMLLLVATEMEPMVVEARTCESQSQRFKGACVSKTNCASVCQTEGFHGGHCRG FRRRCFCTKHC
		Eucgr.C02231.1.p	MIEMPKVEAYCSEGIGLCGKGNECEQRCYASHGPGSKGSCDYNIKPPLCTCYYDCPTPPKPKVCIGGL GLCSNACSNQCCSNRCAAKFSQGRGYCDNSAGPWLCQCQYPC
		Eucgr.H04400.1.pX	MAKYFTLCFLLLLILSCDEKRVPLAEAKDCHKVWTCKGGDKCWQDCKNQFNGRGMCDLYTAPPVP KQCFCAYKC

AMP	Species	Accession Number	Sequence query
		Eucgr.H05052.1.p	MEKSKMRCMGLFMMVLLVLAAQEAEGRVCESQSHGFHGSCVSGHNCALVCRNEGFSGGRCRGFRR RCFCTKLC
		Eucgr.K01859.1.p	MQQSKSRPSIALFSEALSSSPASARTKSQRRKAVSGSAMATKLPLSSLFLLLVLLLSVAWMPPKASAQ PRTCSIELDSTGCRPQDCVARCAAQYRGIGECAHQEYVSNFHCVCLYACL
		Eucgr.C02268.1.p	MARLLQAYCLVLLFVLTAGLMVKTTAAKDCTEGLGTCAGGNDCEQSCFERHGPGSQGTCDRTISPPL CSCIFMCPLST
	Feijoa sellowiana	TRINITY_DN119808_c0_g1_i1 (este estudio)	MKRISICSVLLLVLLLIAGSGSMMAQVSGLCSKKLQLPAHRCIKDECISYCKTTYGDGATGVCESDTQP EDICLCQYPC
	Nicotiana alata	Q8GTM0 · DEF_NICAL	MARSLCFMAFAILAMMLFVAYEVQARECKTESNTFPGICITKPPCRKACISEKFTDGHCSKILRRCLC TKPCVFDEKMTKTGAEILAEEAKTLAAALLEEEIMDN
	Petunia hybrida	Q8H6Q1 · DEF1_PETHY	MARSICFFAVAILALMLFAAYDAEAATCKAECPTWDSVCINKKPCVACCKKAKFSDGHCSKILRRCL CTKECVFEKTEATQTETFTKDVNTLAEALLEADMMV
		Q8H6Q0 · DEF2_PETHY	MARSICFFAVAILALMLFAAYETEAGTCKAECPTWEGICINKAPCVKCCKAQPEKFTDGHCSKILRRC LCTKPCATEEATATLANEVKTMAEALVEEDMME

Table S2. Primers used for validation of the selected sequences

	F	R
AsDf1	TCAACCAAATAGCAGAAAACAAACA	AATCCTTTTTCAGATTGGTATTTGCCT
AsDf2	TCACCACTTCTTCACTCGCC	TGATCACGCCGATGTATCCG
AsDf4	TCAAGAACAAGAAGCCACCACT	TCTCAAAAACCACAATAAAGGGCA
AsDf5	CGTACGAGTTCTCCTGTTTGC	TTCTCGACACGATTGCAAATCT
AsDf6. AsDf7	CCACCACCACCTCCTCAAAG	ACATTACACCCCGGATCAGG
AsSn1	TGACGTGGAACCCATCGTTT	ATCAGCAACAGCTCGGACAT
AsSn13	CACACCCCTTCATTGATCCCA	TGCCCATCCAAATGACACGA
AsSn42	ATTAAGATCGTCGTCGGCGA	GCTTCACATGAACTTTTTGTGCA
AsHv1	TCGTTCGCTTTCCCTATCGT	CCCCTTCTCGATCTCCCTCA

Table S3. Defensins identified in the *Feijoa sellowiana* transcriptome

Name	Sequence*
AsDf1	**MKRISICSVLLLVLLLIAGSGSMMA**QVSGLCSKKLQLPAHRCIKDECISYCKTTYGDGATGVCESDTQPEDICLCQYPC
AsDf2a	**MDFSKRLIPAAFVVMLLLVATEMEPMAVEA**RTCESQSHRFKGACVRDSNCAAVCQTEGFQGGHCRGFRRRCFCTKHC
AsDf2b	**MDFSKRLIPAAFVVMLLLVATGMEPMAVEA**RTCESQSHRFKGACVRDSNCAAVCQTEGFQGGHCRGFRRRCFCTKHC
AsDf3	**MDFSKRLIPAAFVVMLLLVATEMEPMAVEA**RTCESQSHRFKGACVRDSNCASVCQTESFQGGHCRGFRRRCFCTKHC
AsDf4	**MKTHSTSLILLFVMLISFGNELGMKQGAEA**QCQETLYGGGCQDKPCNDACVQKFGPKAYGFCFFYKKPSDTCVCRHPC
AsDf5	**MESLAFPRILVLVLLLSGTCMVMVPQANA**QKRCVEVLDPHSCNLYNCQKACYEKHTAAQQPRGECQHDASFNYSCACFFNC
AsDf6	**MEKSKMRCCMGLFLMLLLVLAEEAEG**RMCQSQSHGFHGSCVRSHNCALVCRNEGFSGGRCRGFRRRCFCTKLC
AsDf7	**MEKSKMRCCMGLFLMLLLVLAAQEAEG**RMCQSQSHGFHGSCVRSHNCALVCRNEGFSGGRCRGFRRRCFCTKLC
AsDf8	MIEIPKVEAYCSEGIGLCGKGNECEQRCYAIRGLGSQGSCDYTIKPPLCTCYYDCPELLPEGYCGSAGSCPEPRLKECNGGGGLCTIACTDQCCSDRCA
AsDf9a	MEPMAVEARTCESQSHRFKGACVRDSNCASVCQTESFQGGHCRGFRRRCFCTKHC
AsDf9b	MEPMAVEARTCESQSHRFKGACVRDSNCASVCQTESFQGGHCRGFRRRCFCTKHC
AsDf10	MLLLFVATAEMEPMVAVEGGRMGKSLSHEYRGICIDRQCAAVCVTEGFWSGYCGGFRRHCICSIPCHPPRPEFHTLLHYILSCLISIMK
AsDf11	**MASGRVCSFIGLLCLISLCFA**EKSVAREINEETVRYVDIGPCSKFPDCNKACKEMLGLNVRGFCKDNGAGSERCYCIVT
AsDf12	LSISDSFVAAWMLPKASAQPRACSVELDTSGCSPQDCVARCASQYSGIGECAHQEYVSNFHCVCLYACL
AsDf13	**MATIKLPLSSLSLLLLLVLLLSAAWMLPKASA**QPRACSVELDTSGCSPQDCVARCASQYSGIGECAHQEYVSNFHCVCLYACL
AsDf14	**MSKTYQICVLLVVLCITMVSVRTQG**TGCLQLSQDFCDVITCDQNCYEFCIAKGCAPQRCRSEGKCSCR
AsDf15	DDCEQRCSAKHGPGSQGTCDAAVSPPLCSCLFSCAPSA
AsDf16	**MAEVFRKCCLLIVLLVFAGWMIEIPKVEA**FCSAGLGLCGKGNDCQQRCSASHGPGSQGSCDYTIN
AsDf17	**MAKYFTLCFLLLLLLLVFPWDEKRVPLAEA**KDCYKVWTCKGGDRCWEDCRNRFNGRG
AsDf18	MELMVAVAGGRKHESLSRKFQGICFSKRNCTAICKPEGFESGRCRGFRRRRFCTRPCPPPSP
AsDf19a	MVAVAGGRKHESLSRKFQGICFSKRNCTAICKPEGFESGRCRGFRRRRFCTRPCPPPSP
AsDf19b	MVAVAGGRKHESLSRKFQGICFSKRNCTAICKPEGFESGRCRGFRRRRFCTRPCPPPSP
AsDf20	MAMVDGKLCQRRSKTWSGFCGNSGNCGRQCRNWEGARNGACHVSSQDS

*The signal peptide sequence is marked in bold.

Table S4. Snakins identified in the *Feijoa sellowiana* transcriptome

Name	Sequence*
AsSn1	**MMKKFALATFLLVALLLSSSFFDAATA**DSSLCDSKCAVRCSKAGRMDRCLQYCGICCEKCQCVPSGTYGHKDECPCYRDMKNSKGKPKCP
AsSn2	**MRFVSVFVCFEGLCDS**KCAVRCSKAGRMDRCLQYCGICCEKCQCVPSGTYGHKDECPCYRDMKNSKGKPKCP
AsSn3	**MKMVQATVFLLACLIVSSSLLDSTMA**AGSSFCGSKCSARCSKAGLKDRCIKYCRICCDKCKCVPSGTYGNKHECPCYRDLKNSKGKPKCP
AsSn4	MKFSRGFCGSKCSARCSKAGLKDRCIKYCRICCDKCKCVPSGTYGNKHECPCYRDLKNSKGKPKCP
AsSn5	TAFCESKCRARCAKAKVKARCAKYCNICCAECKCVPSGTYGNKHQCPCYRDKKNSKGKPKCP
AsSn6	**MKPLFALLLCCLLLTSIFMEPVLA**KPAFCESKCRARCAKAKVKARCAKYCNICCAECKCVPSGTYGNKHQCPCYRDKKNSKGKPKCP
AsSn7	**MKLAFATLLLVSLVLTPSLHAMA**GGLSPQPAPSSCDAKCGGRCQNAGVKDR- CLKYCGICCQKCQCVPSGTYGNKSECPCYRDMKNSKGTAKCP
AsSn8	MRFLLFRPAGSCDAKCGGRCQNAGVKDRCLKYCGICCQKCQCVPSGTYGNKSECPCYRDMKNSKGTAKCP
AsSn9	**MKLAFATLLLASLVLASSLHTMA**GGPSPQPAPSWCDTKCNVRCQRSGWKDRCLKYCGICCQK- CHCVPSGPYVNKSECPCYRDMKNPKGKDKCP
AsSn10	MHIHLSTTLLCTRQSGFQIHFFCFCPTGWCDTKCNVRCQRSGWKDRCLKYCGICCQKCHCVPSGPYVNKSECPCYRDMKNPKGKDKCP
AsSn11	AGVPSIFSRSVSRPRVVLAALEIVVIRRPCVVEYCGNKCEGRCAKAGVMDRCIKYCNICCEECRCVPSGTYGNKHECPCYRDKRSSKGKPKCP
AsSn12	**MKLLLAPALLLCSLLLSFALLGPSSTMS**QYCGNKCEGRCAKAGVMDRCIKYCNICCEECRCVPSGTYGNKHECPCYRDKRSSKGKPKCP
AsSn13	**MSLSKVLACLLLSLLVLNQLVEA**AEATELSSESVESSAAKKIDCGAACAARCRLASRQKICK- RACGTCCARCNCVPPGTAGNRDVCPCYASMTTHGGRLKCP
AsSn14a	SSADCESRCKYRCSKSSRHKMCIRACNTCCQRCNCVPPGTSGNEDVCPCYANMTTHGGRHKCP
AsSn14b	SSADCESRCKYRCSKSSRHKMCIRACNTCCQRCNCVPPGTSGNEDVCPCYANMTTHGGRHKCP
AsSn15	**MASAKTSILFAILCLVLVHELLVFG**GEQFQAEAKKIDCESRCKYRCSKSSRHKMCIRAC- NTCCQRCNCVPPGTSGNEDVCPCYANMTTHGGRHKCP
AsSn16	GLHLRCVDCGGACAARCQLSSRPRLCQRACGTCCARCNCVPPGTSGNLDVCPCYANMTTRGNKRKCP
AsSn17	**MAVHKLLIASILVSVLLLQLAEA**DQMQGVGNAGDSSKLPKMDCG- GACAARCQLSSRPRLCQRACGTCCARCNCVPPGTSGNLDVCPCYANMTTRGNKRKCP
AsSn18	MDCGGACAARCQLSSRPRLCQRACGTCCARCNCVPPGTSGNLDVCPCYANMTTRGNKRKCP
AsSn19	ITNSCPSYEPFSDCGAACAARCQLASRQRLCHRACGTCCNRCNCVPPGTSGNRDVCACYATMTTHGGRLKCP
AsSn20a	MRGFLTDKLPPLQCADCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn20b	MRGFLTDKLPPLQCADCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn21	QLHFSLTKNLQVSSDIDRENSHTQIVKGANRRLLQTLDCGGLCKERCSLHSRPNVCARAC- GTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn22	**LRLSVTSVLLYFSQIVKG**ANRRLLQTLDCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn23a	MRGFLTDKLPPLQCADCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn23b	MRGFLTDKLPPLQCADCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn23c	MRGFLTDKLPPLQCADCGGLCKERCSLHSRPNVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn24	**MALRLLLLVAFLLVCTIQVSS**DIDRENSHTQIVKGANRRLLQTLDCGGLCKERCSLHSRP- NVCARACGTCCFRCKCVPPGTSGNRELCGKCYTDMTTHGNRTKCP
AsSn25	**MAFKAMLFLVATFLLINTRVSS**SDEELKMKAPSPPYAQVPVAAPAPAKLPAKPPTP- SPPSKATPPPAKPPTPTPLPPVKSKADCIPLCDQRCKLHSRKRVCMRACMTCCDRCRCVPPGTYGNREMCGKCYTDMTTHGNRVKCP
AsSn26a	ESDYTELGEPYLVAADCIPLCDQRCKLHSRKRVCMRACMTCCDRCKCVPPGTYGNRERCGKCYTNMTTHGNRIKCP
AsSn26b	ESDYTELGEPYLVAADCIPLCDQRCKLHSRKRVCMRACMTCCDRCKCVPPGTYGNRERCGKCYTNMTTHGNRIKCP
AsSn27	**MASKTVLLLLGLLLLVTTQVSS**DDNEEQLILKAPYAKVPTPSPVKAPAPAKVPVKALPT- LAPVTKTPPPLPPVTKPLPPPLPPVAKPPPTLPPFTKPLPPPLPPVAKPPLPPVAKPPPT- LPPFTKPLPPPLPPVTKPPLPPVANPPPTLPPFTKPLPPPLPPVAKPPLPPVAKPPPT- LPPFTKPLPPPLPPVTKPPPTLPPYTKPLPPPLPPVMKPPPTLPPVMPPAAP- PLPPVRSKADCIPLCDQRCKLHSRKRVCMRACMTCCDRCKCVPPGTYGNRERCGKCYTNMTTHGNRIKCP
AsSn28	MKPPPTLPPVMPPAAPPLPPVRSKADCIPLCDQRCKLHSRKRVCMRACMTCCDRCKCVPPGTYGNRERCGKCYTNMTTHGNRIKCP
AsSn29	**MASKTVLLLLGLLLLVTTQVSS**DDNEEQLILKAPYAKVPTPSPVKAPAPAKVPVKALPT-

Name	**Sequence***
	LAPVTKTPPPLPPVTKPLPPPLPPVAKPPPTLPPFTKPLPPPLPPVTKPPLPPVAKPPPT- LPPFTKPLPPPLPPVTKPPPTLPPYTKPLPPPLPPVMKPPPTLPPVMPPAAP- PLPPVRSKADCIPLCDQRCKLHSRKRVCMRACMTCCDRCKCVPPGTYGNRERCGKCYTNMTTHGNRIKCP
AsSn30a	FTRYLECGPRCKERCSKTAYKKPCMFFCQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn30b	FTRYLECGPRCKERCSKTAYKKPCMFFCQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn30c	FTRYLECGPRCKERCSKTAYKKPCMFFCQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn31	MFPGDDHRGPITTAPGNSRQSARGNVFLEC- QLSDLYIFASVDFFFLFLFFSQYGAREGSLKPQECGPRCKERCSKTAYKKPCMFF- CQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn32	**MARKVSFVILSLFTLLLLIPENQA**TITEAPSPQPQATPGN- LPAYGAREGSLKPQECGPRCKERCSKTAYKKPCMFFCQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn33	LVRKLLCFQATITEAPSPQPQATPGNLPAYGAREGSLKPQECGPRCKERCSKTAYKKPCMFF- CQKCCAKCLCVPPGTYGNKQLCPCYNNWKTKRGGPKCP
AsSn34	TSTGLVGICLQHGTTQGSLKPQDCGPRCTSRCSQTSYKKPCMFFCQKCCAKCLCVPAGTYGNKQTCPCYNNWKTKRGGPKCP
AsSn35	**MAKAQRILVLCI- ALLLVLA**QENEVFGSVELGFDVTVEYDVRASQGSHQMHGTTQGSLKPQDCGPRCTSRCSQTSYKKPCMFFCQKCCAKCLCVPAGTYGNKQT CPCYNNWKTKRGGPKCP
AsSn36	**GLVFLQLFFADCGPRCTS**RCSQTSYKKPCMFFCQKCCAKCLCVPAGTYGNKQTCPCYNNWKTKRGGPKCP
AsSn37	**MAMTKLACVLLLALLGISMVATQVMA**KEAQYHLDSAYGPGSLK- SYQCSGQCSRRCKQTQYKKPCLFFCNKCCAKCLCVPPGFYGNKQVCPCYNNWKTKRGGPKCP
AsSn38	GNSNQKAFDENLTECSGQCSRRCKQTQYKKPCLFFCNKCCAKCLCVPPGFYGNKQVCPCYNNWKTKRGGPKCP
AsSn39	LVEQQVHGKLRPSDCPKKCKFRCSATSHKKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNSWKTKEGGPKCP
AsSn40	**MARRPYTSFLVLSLLLLIALSNVAEVHG**KLRPSDCPKKCKFRCSATSHKKPCMFF- CQKCCAKCLCVPPGTYGNKQVCPCYNSWKTKEGGPKCP
AsSn41	AFNAKTLTYDVVDCPKKCKFRCSATSHKKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNSWKTKEGGPKCP
AsSn42	**MASFSLLRSSLLFFLVASMCFVEVSLA**GGEGSLRPDQCAAACDYRCSETSHRKPCLFF- CNKCCAKCLCVPSGTYGHKEECPCYNNWKTQEGKPKCP

*The signal peptide sequence is marked in bold.

Table S5. Thionins identified in the *Feijoa sellowiana* transcriptome

Name	Sequence*
AsTn1	**MDKVKSVFVVCLVLGLFVGQPSAG**GIKPCYKRCYFHCVIDPRRSLPYCHDKCMKHCLPPSSD-NLKDTRYFCKLGCAYSSCTTFSSKDDPSVRGMP
AsTn2	**MDKVKSAFVVCLVLGLFLGQSRA**DFEDCYQICFILCAITPGNSLF-SCSVKCLKDCIVPPSDSLGDTQYFCKLGCAASSCT-NLSSKDNPGNKSLSLSLSLSLSLYVCVCVCGSSYFCSCLCRSGEKRVAGCVDSCSETCANHV
AsTn3	**MDKVKSAFVVCLVLGLFLGQSRA**DFEDCYQICFILCAITPGNSLF-SCSVKCLKDCIVPPSDSLGDTQYFCKLGCAASSCT-NLSSKDNPGNKSLSLSLSLSLSLYVCVCVCGSSYFCSCLCRSGEKRVAGCVDSCSETCANHV
AsTn4	**MDKVKSAFVVCLVLGLFLGQSRA**DFEDCYQICFILCAITPGNSLF-SCSVKCLKDCIVPPSDSLGDTQYFCKLGCAASSCTNLSSKDNPGEKRVAGCVDSCSETCANH
AsTn5	**MEIKPFDKIMTLFLMMMLLGVSS**QESQSTDCISHCVLS-CANGHVALPLSCFYDCLKECQDNSPNPAPINQCDLDCVTETCLDDDTDARKARVCADECSDICKWPSKPH
AsTn6	**MDVKSCAVLVVLMLVATIALQASA**TEVKSFKSDLCKVQCLVDCAAVPNKRACYLT-CYRNCVGASEVETFESQCNLDCIVSKCFDLLPDVKKVEGCVSSCSKNCKRS
AsTn7	**MEVEASKKMVMMLMVGLIISRSFVEITA**TDCDKLCRDFCAGS-FTPSFCYLICMRGCVKRDALVPQELGHFQLGCVASTCIDHRSDVKKVEADKEKAEACVDSCSENCQKSYGPH

*The signal peptide sequence is marked in bold.

Table S6. Hevein-like proteins identified in the *Feijoa sellowiana* transcriptome

Name	Sequence*
AsHv1	**MRISTLAFLSLVSGALA**GNCGPDNGNKKCAATECCSQYGWCGTTKDHCDLPTCIKAFSGASS- PCEPSGSTPTTTLKTSVVKQATSPATTFPSTVPNIDVCGSAQGGVTCPGVGADGYFYRCCS- SAGHCGPKNDIQDQALYCGDGCQAGFGKCDSMAGPPVPTQTPGTAGDGETCGPIVNRK- CGGSLCCSGSNFCGSGVDFCGAANWCQSKWGRCD
AsHv2	**ISKSYITSVGRRRSSSTMRTTILGVSALIGLATA**NISFT- WVQPTCDITSDFDPCLTGQHCTEENTCAADIHSDRSKVSARSRSSIRFVPEKRQAQYSLD- GKCGPANGNLLCDPNSVNYQGTCCSEYGWCGNTPTHCGTGCQSGCNNGAAPTTAQ
AsHv3	**MHFSNALAVLVASPLVAA**HSGIPGAPKIFGLPRDSKAGAPVVPRVIRHPTEPKG- GLETRQGGQNGRCGPNFGGASCAAGYCCSGAGYCGTTKDHCAAPDCLLDY- GPGCDANTTPSGASTRNDPRPQKGNVAYGGEGVYTCQTPGTVAITYDDGPYSFT- NGVLDQFASYGFKATFFVTGINIG- KGAIDTTASWSNVIKRMVAEGHQVASHTWSHQDLSAITDAQRYDQMVKNEMAIRNII- GKYPTYMRPPYSSCNDACQNVMKNLGYVVSYFDLDTDDYNQLSNIQVAQNNFKTILDQTEGG- SASGDRLAIAHDIHEVSANQLTAYMLSYLKERGYR- GVTMGECMGEPESNWYRTSGEGGGSTTPPPTTGGSVSTDGTCGVQGGKTCTNSSFGNCCSAF- GWCGSSTDHCGTGCNSSFGTCSNGGGSTTPTPAPSKPVSTDGTCGTQGGATCAGSTFGDCCSQYGWCGSSTGHCGSGCDSSSGTCS
AsHv4	MHIPLIHHYKYPSLSSSKLQSNIPPFKMMTTLKLKKLLLTAAALTAIVVTALPHTA- SAQNCGCSADQCCSQWGYCGTGDDYCGTGCQAGPCNAPPPTNGVSVADVVTEAFFDGIIGE- AAESCEGKGFYTREKFLEAVGSYPQFGKVGSVDDSKREIAAFFAHVTHETIHFCYIEE- IDGPSKDYCDEKNTQYPCNPNKGYYGRGPIQLSWNFNYGPAGESIGFDGLNSPETVATDPLI- AFKTALWYWMNFVRPVINQGFGATIRVINGALECDGGNPATVQARVQYYTEYCNQFGVAPGDNLTC
AsHv5	**MAKSVKIARALLNVLVVSGLIAGFLPAHVFG**QNCGCAA- DLCCSQYGYCGTGDQYCGTGCQSGPCSSSSTPTTPSSGTSVADVVTDSFFNSIIGQAAAS- CAGKSFYTRQAFLDAAGAYPEFGTVGSADDSKREIAAFFAHVTHETGRKNLNKMFKVL
AsHv6	**MAKSVKIARALLNVLVVSGLIAGFLPAHVFG**QNCGCAA- DLCCSQYGYCGTGDQYCGTGCQSGPCSSSSTPTTPSSGTSVADVVTDSFFNSIIGQAAAS- CAGKSFYTRQAFLDAAGAYPEFGTVGSADDSKREIAAFFAHVTHETGHFCYIEEIDGAS- KDYCDETNTQYPCNPNKGYYGRGPIQISWNYNYGPAGQSIGFDGLGSPETVAN- DAIVSFKTAFWFWMNNVHSIITSGQGFGATIRAINGGECGGGNSGAVQARVQYYTDYCNQLGVSPGDNLTC
AsHv7	**MDRNALTDILVACLAVLALVVPHVTA**QNCGCAAGLCCSRYGYCGTSHDYCGPGCQAGPCD- PAAAPPATNAVVVGNIVTDAFFNGIINQAAASCAGKRFYSRKAFLDAIGSFPRFGRVGSVDN- SKREIAAFFAHVTHETGHFCYIEEIDGRTDPKKTYCDPNVPQYPCKPGKKYFGRG- PLQISWNYNYGPAGESLGFDGLNAPETVANNPVVAFKTALWFWRTNDLQSKISSQGFGATI- RAINGMECNGGNPGAVQARVTYYNNYCRQFGVAPGGNLSC
AsHv8	HKEGALEKRQGDAQCGAGIGSCAAGQCCSQAGYCGTDPDYCYSPGCNYQYGPGCPDNAV- PSGTNTSTIARTQLGSVAVGGEGIYNCQQVGTIALTYDDGPAKTLTAHILDLMKSYNA- KATFFITGNNINKGQIDITQEYIDVIKRMDSEGHQIASHTWTHLDLSAISQLDRKNQMW- KNEMALRNIVGKIPTYMRPPYSSCTGQCETDMAALGYHVIYFDVDTDDYNQDDPTLIQH- SKDWFKGNITAGGATAAKNTWLDIQHDIHVQTANNLTEFMLQTITSLG
AsHv9	TTDHCGTGCQTGFGTCSGSAVVSSAAASPSAVKTSSAAAASASAAKKVSTDGSCAG- TSGFTCSGSSFGNCCSQYGWCGTTTGHCGTGCQTSFGTCT
AsHv10	QISTSGKCGVANGGLTCTGSSFGECCSQFGFCGRSSAHCNTGCQPAFGKGCKNPVGSIGGLQISPDGSCGGGTGYTCQGSQFGNCCS
AsHv11	PECGEGIGSCPAGKCCSRAGYCGTDDAFCYSPGCKYQYGPGCPENNPPSGTNTSSVTRDKLG- SIAYGGDGIFRCVTPGTVALTFDDGPQTNFTDRILDIFKSYDAKGTFFVTGN- NINKGQIDLKHAATLKRIDAEGHQVASHTWTHLDLSQLSSLDRKNQMWMNEMAIRNVLG- KIPTYMRPPYSSCTKDSGCQQDMADLGYHITNFNVDTDDYNQITAENIQKSKDWFKGNITKDGATSQKGDKWLSISHDIIEQTAN
AsHv12	VKSSAGVSATSSANKVSTDGTCAGTGKQTCQGSTFGNCCSQYNYCGSTGDHCGTGCQSGFGTCGSSSASKAAAVTTTKATPTNATTS

*The signal peptide sequence is marked in bold.

Table S7. LTPs identified in the *Feijoa sellowiana* transcriptome

Name	Sequence
AsLTP1	MACSKSTASLALFFTLNLLFFALVSSCGTCP- SPRPRPRPRPRPRPRPTPTPTPTPVTPTPTPVTPTPSTGACPRDALKLGI- CADVLGSLLNVTIGTPPVTPCCSLLAGLVDLEAAVCLCTAIRANILGINLNIPLSLTLLLNVCSRTVPPGFQCA
AsLTP2	MASRTLATTALLLSLNLLFFTMVSSTYCPPPPPKHHPKHPPKPTPTPTPAKATCPKD- TLKLGVCADVLKSLLHLVVGTPPKTPCCSLIGGLADLEAAVCLCTAIKANVLGIHLNVPVSLSLLLNYCGKKVPSGFQCA
AsLTP3	MASKTYAALALVLSLNIVFASLVAAGPPKLISNSGASCPRDALKLGI- CANLLGSLLNITIGTPPKEPCCSLIAGLADLEAAVCLCTAIKANVLGINLNVPLSLSLLLNVCTKKVPPGFQCA
AsLTP4	MASKTVATGAILLVNLLFFTMVSSTHVPCLPPPPKTLKHPSPSSPAAAACPKD- TLKLGVCANLLNDLLHLVVGTPPKTPCCSLIEGLADLEAAVCLCTAIKANILGINLNVPISLSLLLNYCGKGVPKGFQCA
AsLTP5	MARSTPILILSLLLLAAVSNACNTCMLPSPPPPTSCPPTPATACPRD- TLKLGVCVDLLGGVVGVVVGTPASSPCCALLKGLAGLDVALCLCTAIKANVLGINLNVPIALSTIVSACGESIPPGFKCE
AsLTP6	TPRRIPAAQASSGVPVPCPPPPPAPAAPQTCPIDTLKLGACVDVLG- GLVHVGIGSSAKDTCCPVLQGLLDLDAAVCLCTTIRAKLLNISIVIPIALQVLVDCGKTTPPGFQCPA
AsLTP7	MFPKCPRTTVTVLVLLNIIFFTCVSSHNLPCPPKVPSPSPPPPAPEGLGKCPKDKLKFGVCG- SWLGLVTENIGAKPSKECCTLVAGLADLEAALCLCTAIKANVLGLVKIKVPVALSLLVNGCGKKVPQGFVCA
AsLTP8	MGSETMATFFISMLLLLLSLPPIYACGYCAPPRPPY- HGPTIPRRPGPRPHPGPRHHGGKSGGSGGSRGGGGGRGGGRGG- GYTPRVPTPHPPVVLPPIVNPPPVTNPPGIVPPITNPPGGIVPPIINPPGILPPIT- NPPPTPPSSPCPPYGGSPPGGSKGGGVPNPPTTPTTCPINALKLGLCLDVLGGLVHIGIGN- PIENVCCPVLQGLLELEAAICLCTAIRLKLLNLNIFIPLALQVLATCGITPPPGFVCPPL
AsLTP9	MGSETMATFFISMLLLLLSLPPIYACGYCAPPRPPY- HGPTIPRRPGPRPHPGPRHHGGKSGGSGGSRGGGGGRGGGRGG- GYTPRVPTPHPPVVLPPIVNPPPVTNPPGIVPPITNPPGGIVPPIINPPGILPPIT- NPPPTPPSSPCPPYGGSPPGGSKGGGVPNPPTTPTTCPINALKLGLCLDVLGGLVHIGIGN- PIENVCCPVLQGLLELEAAICLCTAIRLKLLNLNIFIPLALQVLATCGITPPPGFVCPPL
AsLTP10	MGSETMATFFISMLLLLLSLPPIYACGYCAPPRPPY- HGPTIPRRPGPRPHPGPRHHGGKSGGSGGSRGGGGGRGGGRGG- GYTPRVPTPHPPVVLPPIVNPPPVTNPPGIVPPITNPPGGIVPPIINPPGILPPITNPPG- GIVPPIINPPGILPPITNPPPTPPSSPCPPYGGSPPGGSKGGGVPNPPTTPTTCPI- NALKLGLCLDVLGGLVHIGIGNPIENVCCPVLQGLLELEAAICLCTAIRLKLLNLNIFIPLALQVLATCGITPPPGFVCPPL
AsLTP11	MGSETMATFFISMLLLLLSLPPIYACGYCAPPRPPY- HGPTIPRRPGPRPHPGPRHHGGKSGGSGGSRGGGGGRGGGRGGGYTPRVPTPHPPVVLP- PILNPPPVTNPPGIVPPITNPPGGIVPPIINPPGILPPITNPPPTPPSSPCPPYGGSPPGG- SKGGGVPNPPTTPTTCPINALKLGLCLDVLGGLVHIGIGNPIENVCCPVLQGLLELE- AAICLCTAIRLKLLNLNIFIPLALQVLATCGITPPPGFVCPPL
AsLTP12	MDSSKIHALSLILMLCLSSAAPILGCGSCGKAAPKHKKPGHKHHPKGPIVVVPPITLPPIV- KPPVTLPPIVKPPITLPPIVKPPITLPPVTVPPITVPPVINPPVAKPCPPPPATCPIGTLKL- GACVDLLGGLVHIGLGDPVVNECCPVLQGLVELEAAVCLCTTLKLKLLNLSIYVPLALQLLVTCGKTPPPGYTCSL
AsLTP13	MDSSKIHALSLILMLCLSSAAPILGCGSCGKAAPKHKKPGHKHHPKGPIVVVPPITLPPIV- KPPVTLPPIVKPPITLPPIVKPPITLPPVTVPPITVPPVINPPVAKPCPPPPATCPIGTLKL- GACVDLLGGLVHIGLGDPVVNECCPVLQGLVELEAAVCLCTTLKLKLLNLSIYVPLALQLLVTCGKTPPPGYTCSL
AsLTP14	MDSSKIHALSLILMLCLSSAAPILGCGSCGKAAPKHKKPGHKHHPKGPIVVVPPITLPPIV- KPPVTLPPIVKPPITLPPIVKPPITLPPVTVPPITVPPVINPPVAKPCPPPPATCPIGTLKL- GACVDLLGGLVHIGLGDPVVNECCPVLQGLVELEAAVCLCTTLKLKLLNLSIYVPLALQLLVTCGKTPPPGYTCSL
AsLTP15	MESNHLILVFLIWASI- ACLSSTSNLPSEYSIVGENPEKSLLLPEGRVLELFRRWQEKHHKVYKHAAEAEKRLEN- FRKNLRHVIERSSRQKSPAASRVGLNKFADLSNEEFREVYLSKVKKPASKKWN- HKKMSLKGKMKTCDAPSSLDWRNYGIVTAVKDQGECGSCWAFSSTGAMEGINALVNG- DLVSLSEQELVSCDTTNYGCEGGYMDYAFEWVINNGGIDSEADYPYTSSDGETGSCV- TSKEENKVVSIDGYEDVAESDSALLCAVVQQPISVGMDGSALDFQLYTG- GIYNGSCSDDPDDIDHAVLIVGYGSESGEDYWIVKNSWGTDWGIDGYFYILRNTDIE- YGVCAINAMASYPTKESSSAPSPTSPPTPPSPATPPPPPPPVTPPPPPPPPP- SPSPSECGDFSYCPTDETCCCIFEFFDYCLVYGCCEYENAVCCTGTEYCCPSDYPICDVDEGLCLKVWKSPSSSF
AsLTP16	MESNHLILVFLIWASI- ACLSSTSNLPSEYSIVGENPEKSLLLPEGRVLELFRRWQEKHHKVYKHAAEAEKRLEN- FRKNLRHVIERSSRQKSPAASRVGLNKFADLSNEEFREVYLSKVKKPASKKWN- HKKMSLKGKMKTCDAPSSLDWRNYGIVTAVKDQGECGSCWAFSSTGAMEGINALVNG- DLVSLSEQELVSCDTTNYGCEGGYMDYAFEWVINNGGIDSEADYPYTSSDGETGSCV- TSKEENKVVSIDGYEDVAESDSALLCAVVQQPISVGMDGSALDFQLYTG- GIYNGSCSDDPDDIDHAVLIVGYGSESGEDYWIVKNSWGTDWGIDGYFYILRNTDIE- YGVCAINAMASYPTKESSSAPSPTSPPTPPSPATPPPPPPPVTPPPPPPPPP- SPSPSECGDFSYCPTDETCCCIFEFFDYCLVYGCCEYENAVCCTGTEYCCPSDYPICDVDEGLCLKVWKSPSSSF
AsLTP17	MESNHLILVFLIWASI- ACLSSTSNLPSEYSIVGENPEKSLLLPEGRVLELFRRWQEKHHKVYKHAAEAEKRLEN- FRKNLRHVIERSSRQKSPAASRVGLNKFADLSNEEFREVYLSKVKKPASKKWN- HKKMSLKGKMKTCDAPSSLDWRNYGIVTAVKDQGECGSCWAFSSTGAMEGINALVNG-

Name	Sequence
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AsLTP18	MESNHLILVFLIWASI- ACLSSTSNLPSEYSIVGENPEKSLLLPEGRVLELFRRWQEKHHKVYKHAAEAEKRLEN- FRKNLRHVIERSSRQKSPAASRVGLNKFADLSNEEFREVYLSKVKKPASKKWN- HKKMSLKGKMKTCDAPSSLDWRNYGIVTAVKDQGECGSCWAFSSTGAMEGINALVNG- DLVSLSEQELVSCDTTNYGCEGGYMDYAFEWVINNGGIDSEADYPYTSSDGETGSCV- TSKEENKVVSIDGYEDVAESDSALLCAVVQQPISVGMDGSALDFQLYTG- GIYNGSCSDDPDDIDHAVLIVGYGSESGEDYWIVKNSWGTDWGIDGYFYILRNTDIE- YGVCAINAMASYPTKESSSAPSPTSPPTPPSPATPPPPPPPVTPPPPPPPPP- SPSPSECGDFSYCPTDETCCCIFEFFDYCLVYGCCEYENAVCCTGTEYCCPSDYPICDVDE- GLCLKNSGDYLGVAAKRRKMAKHKFPWTKIEETQKSYHPLQWKRNRFAAAMR
AsLTP19	MASYPTKESSSAPSPTSPPTPPSPATPPPPPPPVTPPPPPPPPP- SPSPSECGDFSYCPTDETCCCIFEFFDYCLVYGCCEYENAVCCTGTEYCCPSDYPICDVDE- GLCLKNSGDYLGVAAKRRKMAKHKFPWTKIEETQKSYHPLQWKRNRFAAAMR
AsLTP20	MASYPTKESSSAPSPTSPPTPPSPATPPPPPPPVTPPPPPPPPP- SPSPSECGDFSYCPTDETCCCIFEFFDYCLVYGCCEYENAVCCTGTEYCCPSDYPICDVDEGLCLKVWKSPSSSF
AsLTP21	MLDLRKVSPLLLFLTFLIFGFGTPSTTSAATAAADKSGASTTPALAAVVSRDQVTCT- MCAACENPCQPVPSPPPPPPEVECPPPPSPQPPPSALPPPSPPPPEAECPPPPPMPPAC- GACESPGTGSIYTSPPPPATSGGGGGGVYPPPYGGYYPMQPPPNPIVPYFPYYYYNPPQASVASSSLHLETTAAGFATSLVLSGLLYVLLLRF
AsLTP22	MAPSALLKLACVLLACMVAAAPAARAAVTCGQVTGALAPCIPYARSGSGPVPASCCNG- IRSLNSAAQTTPDRRATCRCLKSASGSISGLNYGVVSGLPGKCGVSIPYKISPSTNCDSVK
AsLTP23	MARYGLSRLVCVTLLCLVVASSVAEAAVTCGQVASALAPCISYARSGRGPVPAGCCNGIRSLN- NAARTTPDRQATCRCLKAAAGGISGINYGVVAAIPGKCGVSIPYKISPSTNCNSVK
AsLTP24	MAPSALLKLACVLLACMVAAAPAARAAVTCGQVTGALAPCIPYARSGSGPVPASCCNG- IRSLNSAAQTTPDRRATCRCLKSASGSISGLNYGVVSGLPGKCGVSIPYKISPSTNCDRYVLF
AsLTP25	MAPSALLKLACVLLACMVAAAPAARAAVTCGQVTGALAPCIPYARSGSGPVPASCCNG- IRSLNSAAQTTPDRRATCRCLKSASGSISGLNYGVVSGLPGKCGVSIPYKISPSTNCDRYVILVSRSEGVTKPYFSL
AsLTP26	MADLSKLSCFLLLACLVAPLQPIGSAAVTCSQVSTSMAPCIGYLRGGGPLPDACCSGVQSLN- GAARTTADRQAACRCLQSAAGSIPGINLGLAAGLPGKCGVNVPYKISPSTNCNSVK
AsLTP27	MARYGLSRLVCVTLLCLVVASSVAEAAVTCGQVASALAPCISYARSGRGPVPAGCCNGIRSLN- NAARTTPDRQATCRCLKAAAGGISGINYGVVAAIPGKCGVSIPYKISPSTNCNRSRYSTKALTYKRH
AsLTP28	MADLSKLSCFLLLACLVAPLQPIGSAAVTCSQVSTSMAPCIGYLRGGGPLPDACCSGVQSLN- GAARTTADRQAACRCLQSAAGSIPGINLGLAAGLPGKCGVNVPYKISPSTNCNS
AsLTP29	MASLKLLCLALVVGLAAVAPLAASGVNCGQVASYVAPCLGYLRVGGLIPQACCNG- IRSLNTAAKDTPNRQATCRCLQAAAGNIRGLKLGLVSALPGKCGINVPYKISPSTDCSRVR
AsLTP30	MANPGLLKTAALAVAVVCMLVANAPPAAAQMSSCNQVVNTLMPCASYVLNGGAVPAACCNG- IRSLYSAAKTTADRQGVCKCLESAINGIPYNAYNAGLAEGLPGKCGVNIPYKISPSTDCKSVQ
AsLTP31	MANPGLLKTAALAVAVVCMLVANAPPAAAQMSSCNQVVNTLMPCASYVLNGGAVPAACCNG- IRSLYSAAKTTADRQGVCKCLESAINGIPYNAYNAGLAEGLPGKCGVNIPYKISPSTDCKRQKHRHFIERH
AsLTP32	MANPGLLKTAALAVAVVCMLVANAPPAAAQMSSCNQVVNTLMPCASYVLNGGAVPAACCNG- IRSLYSAAKTTADRQGVCKCLESAINGIPYNAYNAGLAEGLPGKCGVNIPYKISPSTDCKRQVLHHL
AsLTP33	MKGAALAVALAAVVAVVALMASQVEGITCGQVNKSLGPCAPYLTKGGDPGSACCNGVRELK- NSTPYPADRRAACVCIKELASRVHNIKADAAAALPGKCGVSIGVPIRTDVDCNSIS
AsLTP34	MRPSSFAVLALLVALFLLLARFPVSESAVSCSDVLKDLKPCVSYLKSGSGMPPAACCAGVSA- LANAATSSADKKAACVCIKNAAQKMNPNAQLAQALPGNCKITLPVAVSANVDCSKVG
AsLTP35	MRPSSFAVLALLVALFLLLARFPVSESAVSCSDVLKDLKPCVSYLKSGSGMPPAACCAGVSA- LANAATSSADKKAACVCIKNAAQKMNPNAQLAQALPGNCKITLPVAVSANVDCS- KYVFNLALKFFEKEQIITHREKNLRELPLVLSYASELFGLSF
AsLTP36	MPDGQAACKCLKSASESISGLNYGVVAGLPGKCGVSIPYKISPSTNCDR
AsLTP37	MAARRMEAALVLAAVAMLWVRVAAQSSCTNVLISLSPCLNYITGNSSNP- SPNCCAQLASVVRSQPQCLCQVLSGGGSSLGINVNQTQALALPGACNVQTPPISRCNGKQIIQMMMVFSFKIQKG
AsLTP38	MAARRMEAALVLAAVAMLWVRVAAQSSCTNVLISLSPCLNYITGNSSNP- SPNCCAQLASVVRSQPQCLCQVLSGGGSSLGINVNQTQALALPGACNVQTPPISRCNGKQIIQMMMVFSFKIQKG
AsLTP39	MAARRMEAALVLAAVAMLWVRVAAQSSCTNVLISLSPCLNYITGNSSNP- SPNCCAQLASVVRSQPQCLCQVLSGGGSSLGINVNQTQALALPGACNVQTPPISR
AsLTP40	MTHKVFLTTPPQFTDDNPSPTAAMTAPRSAMLVTVLVAVAVALLWAGAEAQTSCSNALTSL- SPCLSFITGNSSAPSPACCTQLASVVNSEPLCLCAVLNGSASSALGVNINQTQALQLPPAC- NVQTPPTSSCNAALSPAGSPAGSPTSTPGTRASLSLSLP
AsLTP41	MTHKVFLTTPPQFTDDNPSPTAAMTAPRSAMLVTVLVAVAVALLWAGAEAQTSCSNALTSL- SPCLSFITGNSSAPSPACCTQLASVVNSEPLCLCAVLNGSASSALGVNINQTQALQLPPAC- NVQTPPTSSCNAALSPAGSPAGSPTSTPGGSSSDGSSASLRWSLVLFTLFMASHEAVMLMQNNLEPI

Name	Sequence
AsLTP42	MRYVRHGKGSRHCLPTCCSGLQQLARNVQSVNDKQDICRCLKAGAKFVGGLQDKFPSQIPTACHINVGFPVSANVRCEKIHSAVWR
AsLTP43	MGSKTSNGQLAAKVLFFSIIMFMSSNPVNSITCQDALKVVLPCGPFALGAVPPPPSAEC- CSGAQALASMANTTEARRTLCQCYKDIPPSVGIKPERVQEIPQYCKVNVTIPTDPHVDCSSIP
AsLTP44	MAARRMEAALVLAAVAMLWVRVAAQSSCTNVLISLSPCLNYITGNSSNP- SPNCCAQLASVVRSQPQCLCQVLSGGGSSLGINVNQTQALALPGA
AsLTP45	MAARRMEAALVLAAVAMLWVRVAAQSSCTNVLISLSPCLNYITGNSSNP- SPNCCAQLASVVRSQPQCLCQVLSGGGSSLGINVNQTQALALPGA
AsLTP46	LGSIVQSQPRCLCLLLNGTASSLGYNINQTLALALPGACNVNMPSASLCKSPSPSSAPTTSP- PADDNTPVAPTASSTPTVPSGSKTTPTTSGSKGIGVSLGSILFALILASFGSNPLKF
AsLTP47	MALKNLEIMGLALLTVLAAALLARPGLAQSTSGCTQVLMTLTPCLNYVTGNSSAPSSTCC- STLGSIVQSQPRCLCLLLNGTASSLGYNINQTLALALPGACNVNMPSASLCK- SPSPSSAPTTSPPADDNTPVAPTASSTPTVPSGNNLTSLTLLVQQ
AsLTP48	MALKNLEIMGLALLTVLAAALLARPGLAQSTSGCTQVLMTLTPCLNYVTGNSSAPSSTCC- STLGSIVQSQPRCLCLLLNGTASSLGYNINQTLALALPGACNVNMPSASLCKCNGLTSKNYSSFVFWTSVLG
AsLTP49	MGKICY- LIFTSFFLALANCQSPSLVSSSSCSQIIYEMIDCISFLTGAGGSAAKPDALCCSGFKSVLKA- DAHCICQAIREAPQMGIRLNKTRAIELPSDCGVSHFSALKICGCNCVFNSFQ
AsLTP50	MGKICY- LIFTSFFLALANCQSPSLVSSSSCSQIIYEMIDCISFLTGAGGSAAKPDALCCSGFKSVLKA- DAHCICQAIREAPQMGIRLNKTRAIELPSDCGVSHFSALKICGFLD- SPGTPSAESPKTPVPPPTKTPTPSPLPPTMSPASSPRRTKPAPSPTPPPVPSPKSLVSPAP- SPTPSTTNTPVPSPKSPVSSLPKSTSSAPSSLERGISQVPAPAPENLKSGGYSLRACFAVVISLCAIHVSV
AsLTP51	MAQTKMRIVALILAMVAAGAAAQSSSCTNALISMSPCLNY- ITGNSSSPSSSCCSQLANVVRSEPQCLCQALNGGGSSLGISINQTRALAVPSACNIQTQPISRCNGECVCTRKTSKLTSFISSSKSFDLDLTFCTD
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AsLTP53	MAQTKMRIVALILAMVAAGAAAQSSSCTNALISMSPCLNY- ITGNSSSPSSSCCSQLANVVRSEPQCLCQALNGGGSSLGISINQTRALAV- PSACNIQTQPISRCNAASPSASPTGAPDTPNAVPSDSGTKDVPTTERDGSSAGSMAELSIPLLFALLFAASYASANT
AsLTP54	MASKGDEMGLGRVMALVVTMAALCAGA- VAQSGCTSVLMGLAPCLSFVTGSSSTPSSSCCSQLASVVQSQPRCLCMVLNG- GASSSLGVTLNQTLALALPGACKVQTPPVSKCNASGGTVAPAGSPESSPSDVSGGTSSPSGS
AsLTP55	MASKGDEMGLGRVMALVVTMAALCAGA- VAQSGCTSVLMGLAPCLSFVTGSSSTPSSSCCSQLASVVQSQPRCLCMVLNG- GASSSLGVTLNQTLALALPGACKVQTPPVSKCNAGSPESSPSDVSGASKEVSSTAGSSTSHGIAKMPPTLPLAVLSVFMATFGYAPMGMAI
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AsLTP57	MASKGDEMGLGRVMALVVTMAALCAGA- VAQSGCTSVLMGLAPCLSFVTGSSSTPSSSCCSQLASVVQSQPRCLCMVLNG- GASSSLGVTLNQTLALALPGACKVQTPPVSKCNASGGTVAPAGSPESSPSDVSGAS- KEVSSTAGSSTSHGIAKMPPTLPLAVLSVFMATFGYAPMGMAI
AsLTP58	MIDMALKRMQIVLLAAVLAASLARPCSAQSTSGCTAALMTLTPCLSYVSGNPSTPSSTCC- STLSSVVQSQPRCLCLLLNGGVPSLRYNINQTLALALPGACSVKTPSVSLCNAL- SPSSPPTTSPPADSSDNTPVTPTASSTPTIPSGSKATPAATSTSRSIEASFGSLAFALLLASF
AsLTP59	MSDVRQGGSVSDVRHGKGSRHCLPTCCSGLQQLVRNVRSVNDNQDICRCLKAGAKFVGGLQDKFPSQIPTACHINVGFLVSANVSCEKFH
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AsLTP61	MCSFTEKMERRNIRAALETAALVLAALLVVDAQAQDTSCLSRLAPCLNYLNGTRD- PPSTCCDPLKSVISSDPGCLCGLISNQGADQAQQAGINVTEAQQLPGRCGQHVNPIACLSGSPGSRNSDENSAGLFPGSSLRTPLAIVALSVAAQI LWD
AsLTP62	FSNEIHCPVTKCLFFSALFLISLVVSAEATITCSVVNAKVAPCAMYTMGKAPDPASPLAAVACNSSPGTFDQSTTSKTFAVA
AsLTP63	MASRGIMMGLVLVLAATLWTRSAAQSGCTAALTSLVPCLNFINGNLSAPSSSCCSQLASVV- KASPRCLCSVMNGPPMFGITINQTQALALPNACNVQTPPVSQCNAVANAPTPASVGAPEVPP- SPSDSPAGPSDETPTAAAPAATTPPSGTTGTGSKAVPSTNGNTSDGSIVKAQLHLAALAVLAASIVKRF
AsLTP64	MPPMSHVVRTLLGLLLVSAWAAASGHPTCKYVDEYFPDCLDFLVGTYYIPPQRCCRSVGALNY- MANHLVGPRMICQCIEAMVMRTNPPLMASRIESLPKFCATHLSFPISTAMDCSRVT
AsLTP65	MPPMSHVVRTLLGLLLVSAWAAASGHPTCKYVDEYFPDCLDFLVGTYYIPPQRCCRSVGALNY- MANHLVGPRMICQCIEAMVMRTNPPLMASRIESLPKFCATHLSFPISTAMDCSR
AsLTP66	MPPMSHVVRTLLGLLLVSAWAAASGHPTCKYVDEYFPDCLDFLVGTYYIPPQRCCRSVGALNY- MANHLVGPRMICQCIEAMVMRTNPPLMASRIESLPKFCATHLSFPISTAMDCSR
AsLTP67	MAAFFNCWTCYEVPVFLGLVPHFPPRVGRGHHHVQHGQCQGGSVSDVRHGKGSRH- CLPTCCSGLQQLVRNVRSVNDNQDICRCLKAGAKFVGGLQDKFPSQIPTACHINVGF

Name	Sequence
AsLTP68	MVHISIIHLSLFLLLCVSSRGDEFTFCINVYQKFADCMAFLRGLAPVPTGDCCSNLLAL- NDLAKRKQSSPELLCQCNEGIGYVLDTPYLPSRIEDLRSKCQVHLSFPISNAMNCSMGMTRQAKLELTNE
AsLTP69	MVHISIIHLSLFLLLCVSSRGDEFTFCINVYQKFADCMAFLRGLAPVPTGDCCSNLLAL- NDLAKRKQSSPELLCQCNEGIGYVLDTPYLPSRIEDLRSKCQVHLSFPISNAMNCSI
AsLTP70	MESPMGKFICILALLLMASMASRGAHAAGECGKSTTPDAEAMKLAPCAVAAQDA- KAPVSDSCCAQVKSIGKSPSCLCAVMLSNTAKASGINPEIAITIPKRCNIPNRPVGYKCGAYTLP
AsLTP71	MEAPMKQISVLVFFAILAISGFQTVYGAGECGKSSTPDREAF- KLAPCMSAAQDETAPVSSSCCARVKRIGQNPSCLCAAMLSNTAKIAGIKPEVAVTIPKRCNLSDRPAGYKCGGYTVP
AsLTP72	CARVKRIGQNPSCLCAAMLSNTAKIAGIKPEVAVTIPKRCNLSDRPAGYKCGGQISLLSLTAEELSFGNLYLQMHPALDVTML
AsLTP73	MASKASGCSCLVTLVLFLVSSMASREAIAAGECGKTPISTAAVSLSPCLSAAGNAKAKVP- PACCTKVGALIATAPKCLCAVLLSPLAKQAGINLGIAITIPKRCNIKKRPVGKKCGKYTVP
AsLTP74	MSHNSHVAMFVPLSILMVLAISNGFIWVASAAGECGRTPIRSAAASLSPCLGAARDARAKVP- PACCAKTNALIRAAPRCLCAVLLSPLAKQAGITPGTAISIPKRCNIRNRPAGKKCGRYTVP
AsLTP75	MARNSLVPLSILMVLAISNGFIWVASAAGECGRTPVRSAAASLSPCLGAARNARAKVPPAC- CAKVNALIRTAPRCLCAVLLSPVAKQAGIMPGTAISIPKRCNIRNRPAGKKCGRYTVP
AsLTP76	MSHNSHVAMFVPLSILMVLAISNGFIWVASAAGECGRTPIRSAAASLSPCLGAARDARAKVP- PACCAKTNALIRAAPRCLCAVLLSPLAKQAGITPGTAISIPKRCNIRNRPAGKKCGSEYKHPIPNSPLK
AsLTP77	MGEHQTGMKTHSRSYHALDLLKPPLLQPNSPTPHKEFRTQTMATLHY- ISFLPLLLVGIPAALSQEPASSSPAVASCQPRLLSLAPCAPFVQG- TAQSPSEPCCDNLSQIYTQQPHCLCLVLNDTSLVPLPINGTLVLELPQLCNLQADASSCSGNNETFKGTKRIKIGFNPSEPNIFTKGSGFG
AsLTP78	MGEHQTGMKTHSRSYHALDLLKPPLLQPNSPTPHKEFRTQTMATLHY- ISFLPLLLVGIPAALSQEPASSSPAVASCQPRLLSLAPCAPFVQG- TAQSPSEPCCDNLSQIYTQQPHCLCLVLNDTSLVPLPINGTLVLELPQLCNLQADASSCSGL- GAPASPSAPGPQVSVGARRTNNSTVAASPVVEVTPRPSIIRSGPGLSSGTMLDREGRPTFLVAAAVAILLLAQVFY
AsLTP79	MASRGIMMGLVLVLAATLWTRSAAQSGCTAALTSLVPCLNFINGNLSAPSSSCCSQLASVV- KASPRCLCSVMNGPPMFGITINQTQALALPNACNVQTPPVSQCNGKS- FPLAISLCAFCLFSYIFVHLEAVANAPTPASVGAPEVPPSPSDSPAGPSDETPTAAAPA
AsLTP80	MRAPRVALCALLAVLLLAEAGVSVEAAVTCKPIELSSCVSAITSASPPTTLCCSKLKEQRPCLCQYLRNPNLKKFVNSPNARKVASTCGSPFPKC
AsLTP81	MTKPSNRSPSPRLLAAALVAALVLLSLDRAPVAEA- VTCSATELSSCVAAITSSAPPSALCCSKLREQRPCLCGYIRNPNLRQYVTSPNAKRVARTCGVPYPTC
AsLTP82	MKAPSIVVCSLLLLLLAEAQITMAVTCKPTELSSCANSIVSSTPPSSLCCTKIKEQKPCLCQYLKNPSLKKFVNSPNARKVASTCGTPFPKC
AsLTP83	MTKPSNRSPSPRLLAAALVAALVLLSLDRAPVAEA- VTCSATELSSCVAAITSSAPPSALCCSKLREQRPCLCGYIRDPNLRPYATSPNVKRVASTCGVAYPTC
AsLTP84	MSKYVLCIVLIFLFVKLEPSTAVTCDPMELSSCASAISSSSPPSAMCCAKLKEQKPCLCQYMKNPNLQKLINSPNSRKVAIQCGSPFPSC
AsLTP85	MAKPSNRSPSPRLLVAALMAALVLLSPDRAPVAEAVTCTINELSSCLPAV- TSSAPPSALCCSKLREQRPCLCGYIRDPNLRPYATSPNVKRVASTCGVAYPTC
AsLTP86	MTKPSNRSPSPRLLVAALMAALVLLSPDQAPVAEA- VTCSATQLSSCVSAMTSSAPPSALCCSKMREQRPCLCGYIRNPNLRKYLTSADGKRVMHVCGVPYPTC
AsLTP87	MTRKGPSPFVASFLVTALVVSSQLAPGTEAECDATQLTPCLPSLMPPF- SPPTDTCCGKLKEQQSCFCDYMKDPRYGKYVKSPRAKKILELCNIPYPQC

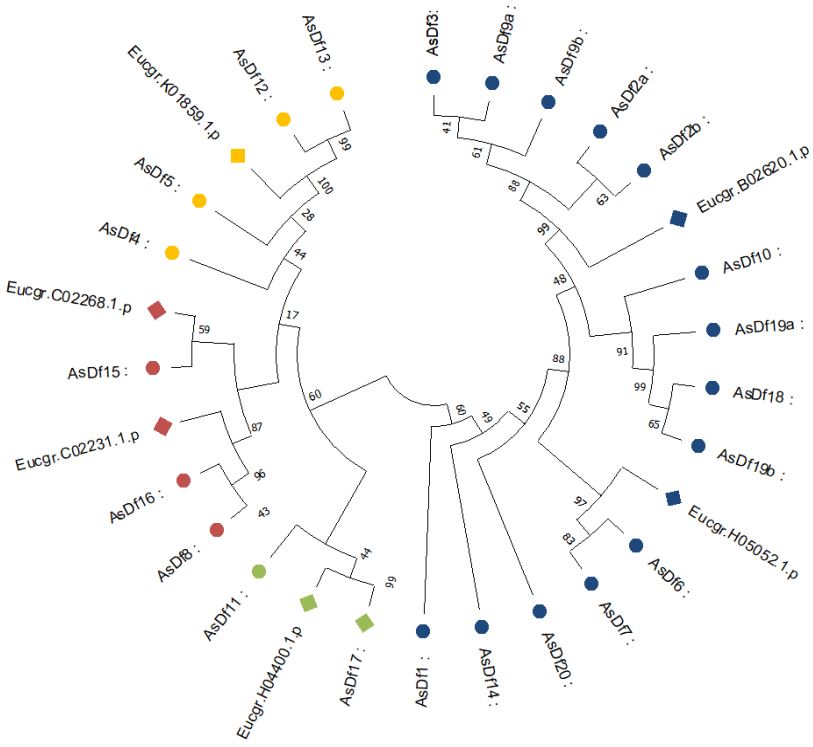
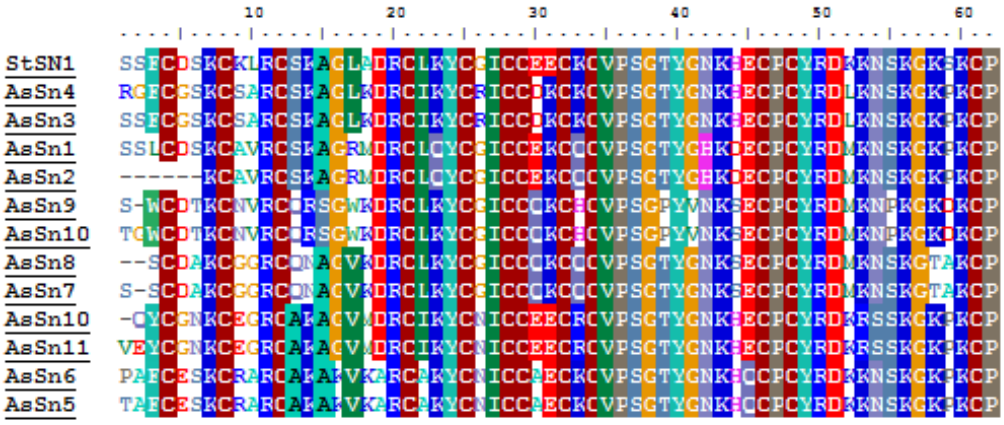


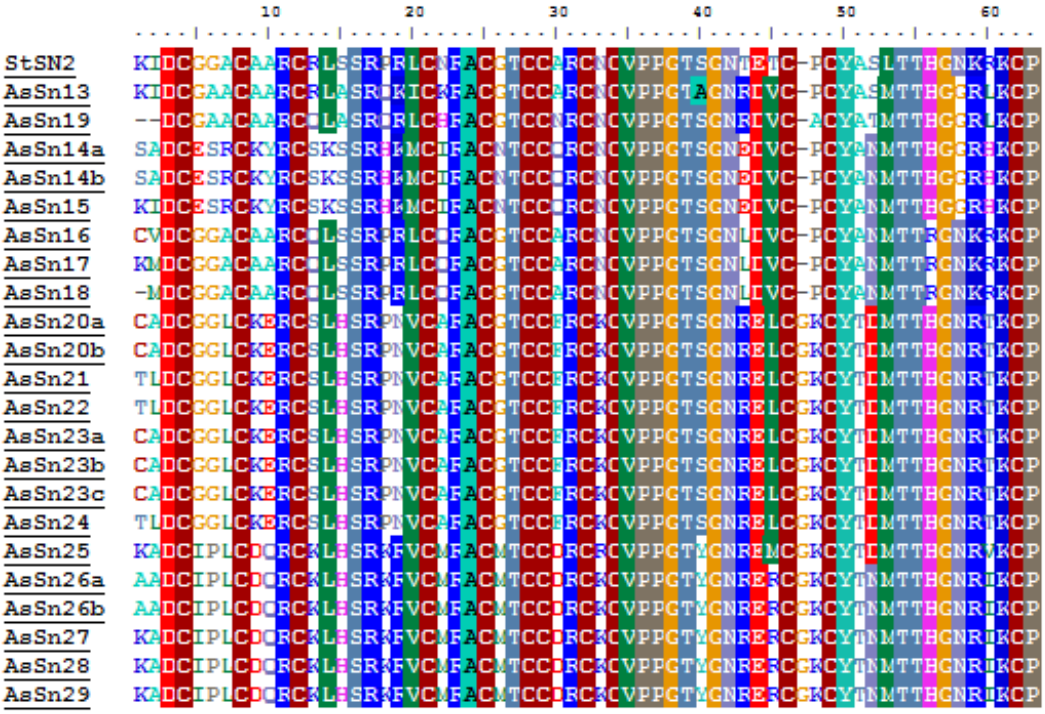
Figure S1. Neighbor-Joining cladogram of putative defensin peptides found in *Feijoa sellowiana* transcriptomes and *Eucalyptus* defensins selected based on amino acid sequence similarity

Diamonds represent *Eucalyptus* defensins, and circles represent *F. sellowiana* proteins. Node values indicate bootstrap values (5000 replicates). Group 1 (blue), Group 2 (green), Group 3 (red), and Group 4 (yellow) are distinguished.

a)



b)



c)

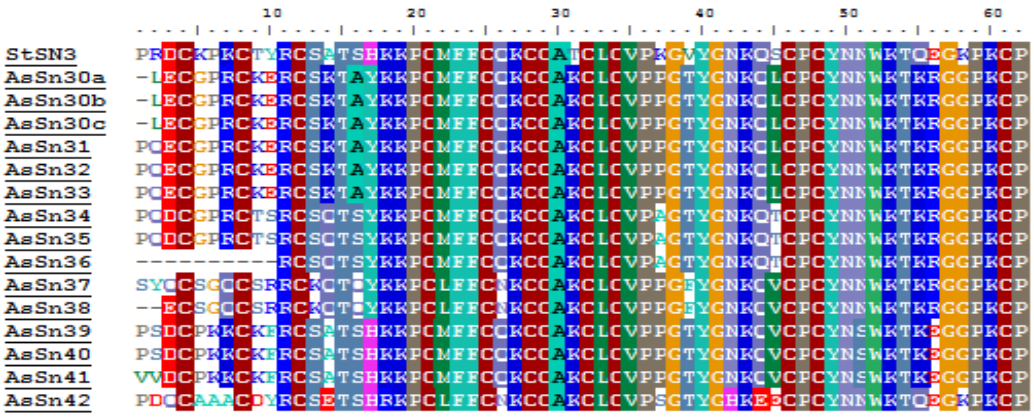


Figure S2. Alignment of the GASA domain of 49 snakins from *Feijoa sellowiana* with reference sequences from *Solanum tuberosum*: a) Subfamily 1, b) Subfamily 2, and c) Subfamily 3, representing the snakin/GASA domains

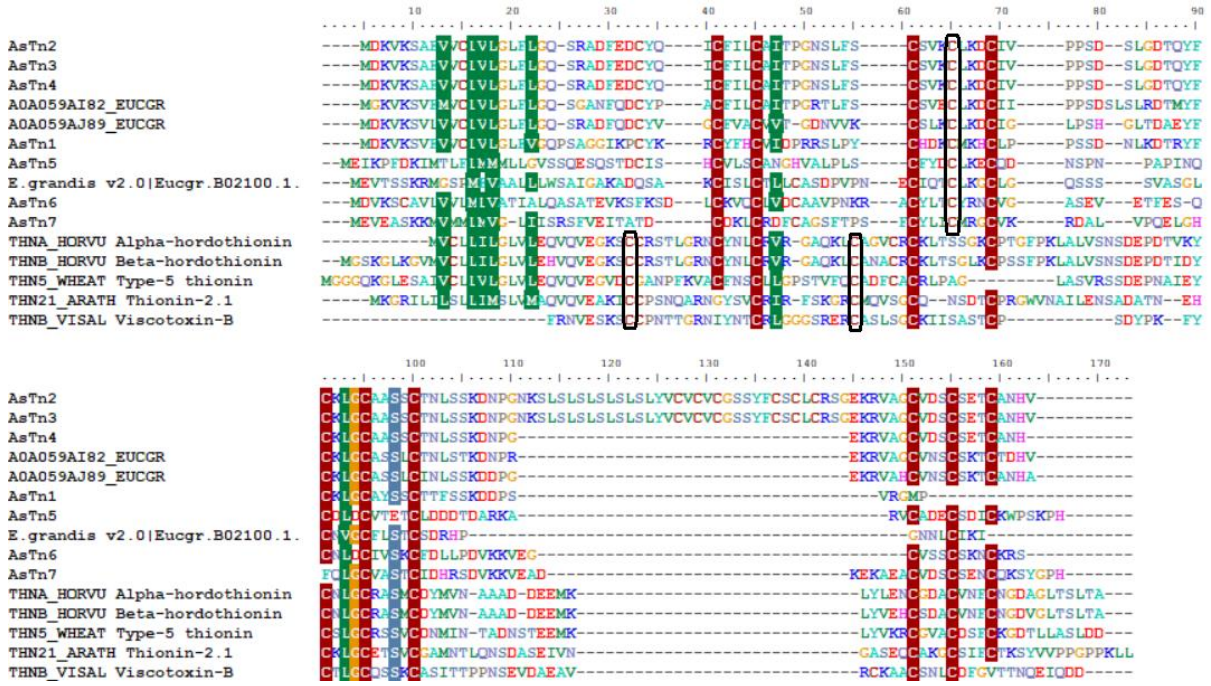


Figure S3. Alignment of seven thionins from *Feijoa sellowiana* with reference sequences from *Eucalyptus grandis*, *Triticum aestivum*, *Hordeum vulgare*, *Arabidopsis thaliana*, and *Viscum album*

Differences in the cysteine patterns of the reviewed sequences, compared to the putative thionins from *F. sellowiana* and *E. grandis*, are marked in black.