Table S1: RNA-seq library summary statistics. UC1-3: chelicerae; UP1-3: prosoma; UG: gonads; UM1-3: midgut glad; US1-3: silk glands.

Libraries	Total raw reads	High quality reads
UC1	58'494'780	48'903'542
UC2	53'421'906	38'774'986
UC3	54'406'220	38'713'640
UP1	55'299'872	46'506'656
UP2	75'303'620	67'291'910
UP3	98'080'052	74'005'606
UG1	107'756'820	96'386'224
UM1	96'262'266	85'748'874
UM2	90'920'880	83'279'616
UM3	95'046'808	86'684'478
US1	53'881'834	44'579'252
US2	53'881'834	48'642'782
US3	53'941'648	49'819'522

Table S2: *De novo* transcriptome assembly summary statistics. ORF: open reading frame.

	# Transcripts	
	Complete ORF	**Incomplete ORF**
Assembled sequences	302'504	
Transcripts with ORF	159'668	66'125
Annotated sequences	33'129	260
Clustered sequences*	20'050	193

* After grouping sequences with 99% identity.

Table S3: List of the 11 spider genomes used for the assembly annotation.

Assembly accession	**Species**	**Total sequence length**	**Assembly level**	**Assembly submission date**
GCA_013235015.1	*Araneus ventricosus*	3'656'621'265	Scaffold	2019-08-02
GCA_015342795.1	*Argiope bruennichi*	1'670'285'661	Chromosome	2020-11-16
GCA_021605075.1	*Caerostris darwini*	1'501'919'382	Scaffold	2021-11-19
GCA_021605095.1	*Caerostris extrusa*	1'420'656'204	Scaffold	2021-11-19
GCA_019974015.1	*Nephila pilipes*	2'694'500'076	Scaffold	2021-07-23
GCA_019343175.1	*Oedothorax gibbosus*	821'427'276	Chromosome	2021-08-05
GCA_000365465.3	*Parasteatoda tepidariorum*	1'228'972'128	Scaffold	2019-06-14
GCA_010614865.2	*Stegodyphus dumicola*	2'551'176'228	Scaffold	2020-02-14
GCA_019973975.1	*Trichonephila clavata*	2'497'895'991	Scaffold	2021-07-23
GCA_019973935.1	*Trichonephila clavipes*	2'874'350'602	Scaffold	2021-07-23
GCA_019973955.1	*Trichonephila inaurata madagascariensis*	2'507'041'000	Scaffold	2021-07-22

Table S4: List of fastq files used to verify and quantify defensin in the genome of *Octonoba sinensis*.

SRA	**Species**	**tissue**
SRR26131816	*Octonoba sinensis*	Brain
SRR26148751	*Octonoba sinensis*	Abdomen
SRR26148754	*Octonoba sinensis*	Brain
SRR26148755	*Octonoba sinensis*	Brain
SRR26148758	*Octonoba sinensis*	Chelicera
SRR26148759	*Octonoba sinensis*	Chelicera
SRR26148770	*Octonoba sinensis*	Silk gland
SRR26148771	*Octonoba sinensis*	Silk gland
SRR26148772	*Octonoba sinensis*	Silk gland
SRR26148773	*Octonoba sinensis*	Gut
SRR26148774	*Octonoba sinensis*	Gut
SRR26148775	*Octonoba sinensis*	Gut
SRR26148776	*Octonoba sinensis*	Abdomen
SRR26148777	*Octonoba sinensis*	Abdomen

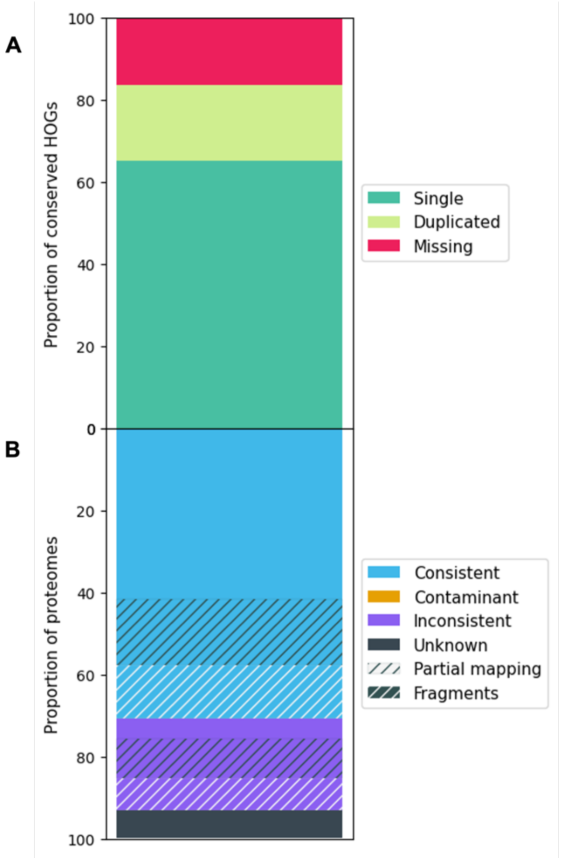


Fig. S1 OMArk completeness evaluation of *U. plumipes* transcriptome assembly. **A**. Proportion of conserved Hierarchical Ortholog groups (HOGs) using Arthropoda as ancestral clade which contains 3’589 conserved HOGs. Results on conserved HOGs: Single: 2’335 (65.06%); Duplicated: 662 (18.45%); Duplicated, Unexpected: 639 (17.80%); Duplicated, Expected: 23 (0.64%); Missing: 592 (16.49%). **B**. Proportion of proteins in the transcriptome with a consistent lineage placement: Total consistent, 14’328 (70.78%); Consistent, partial hits, 3’290 (16.25%); Consistent, fragmented: 2’617 (12.93%). Inconsistent lineage placements: Total inconsistent, 4’512 (22.29%); Inconsistent, partial hits: 1’948 (9.62%); Inconsistent, fragmented: 1’547 (7.64%). Total unknown: 1’403 (6.93%).

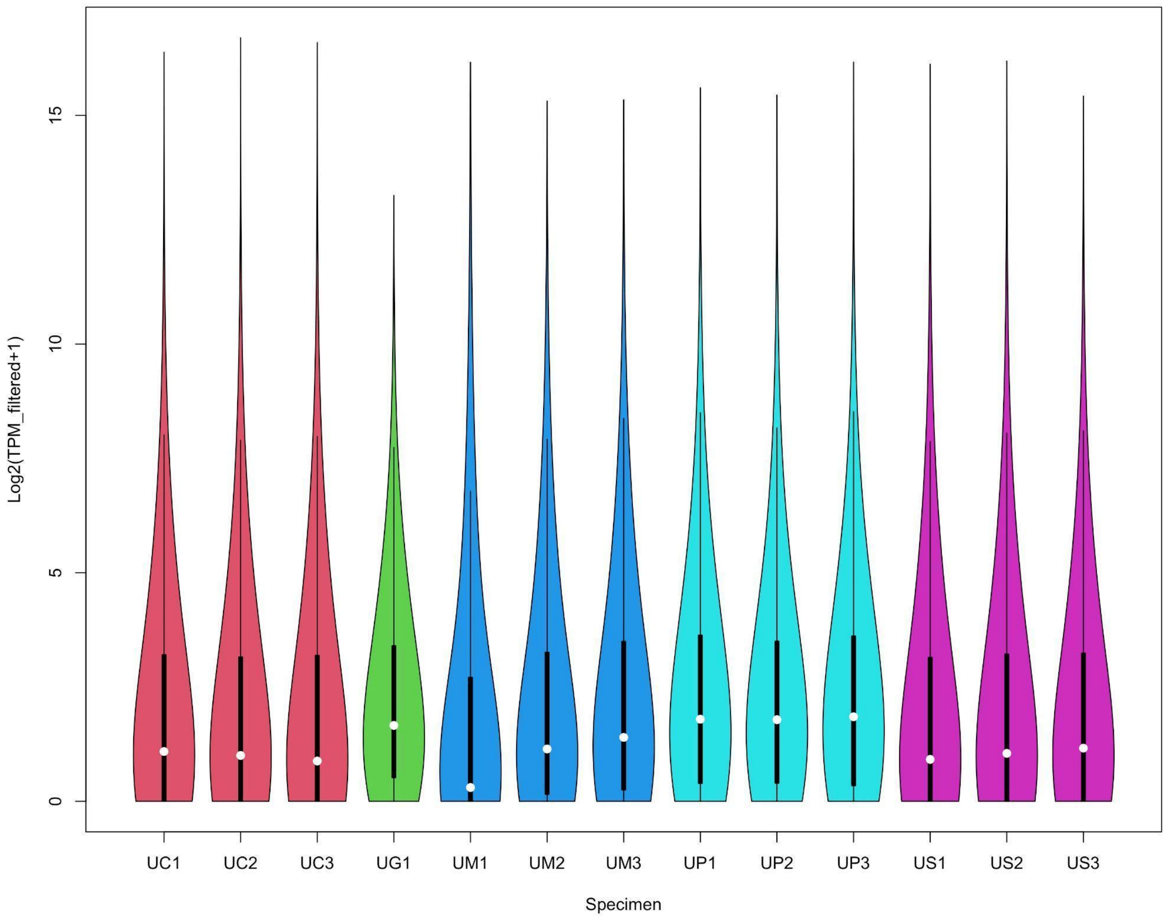


Fig. S2 Violine plot of library transcripts per million (TPMs) data. UC1, UC2, UC3: chelicerae; UG1: gonad; UM1, UM2, UM3: midgut gland; UP1, UP2, UP3: prosoma; US1, US2, US3: silk glands.

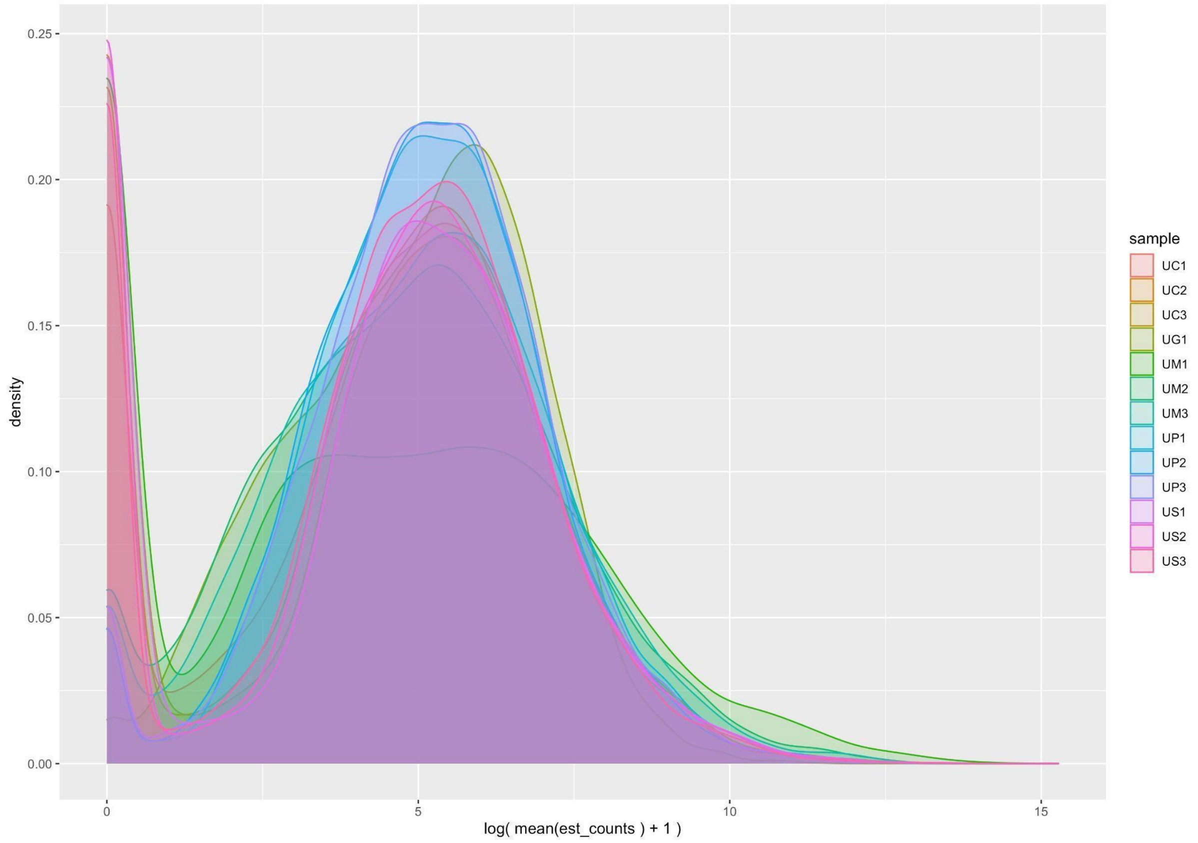


Fig. S3 Read count density of libraries. UC1, UC2, UC3: chelicerae; UG1: gonad; UM1, UM2, UM3: midgut gland; UP1, UP2, UP3: prosoma; US1, US2, US3: silk glands.

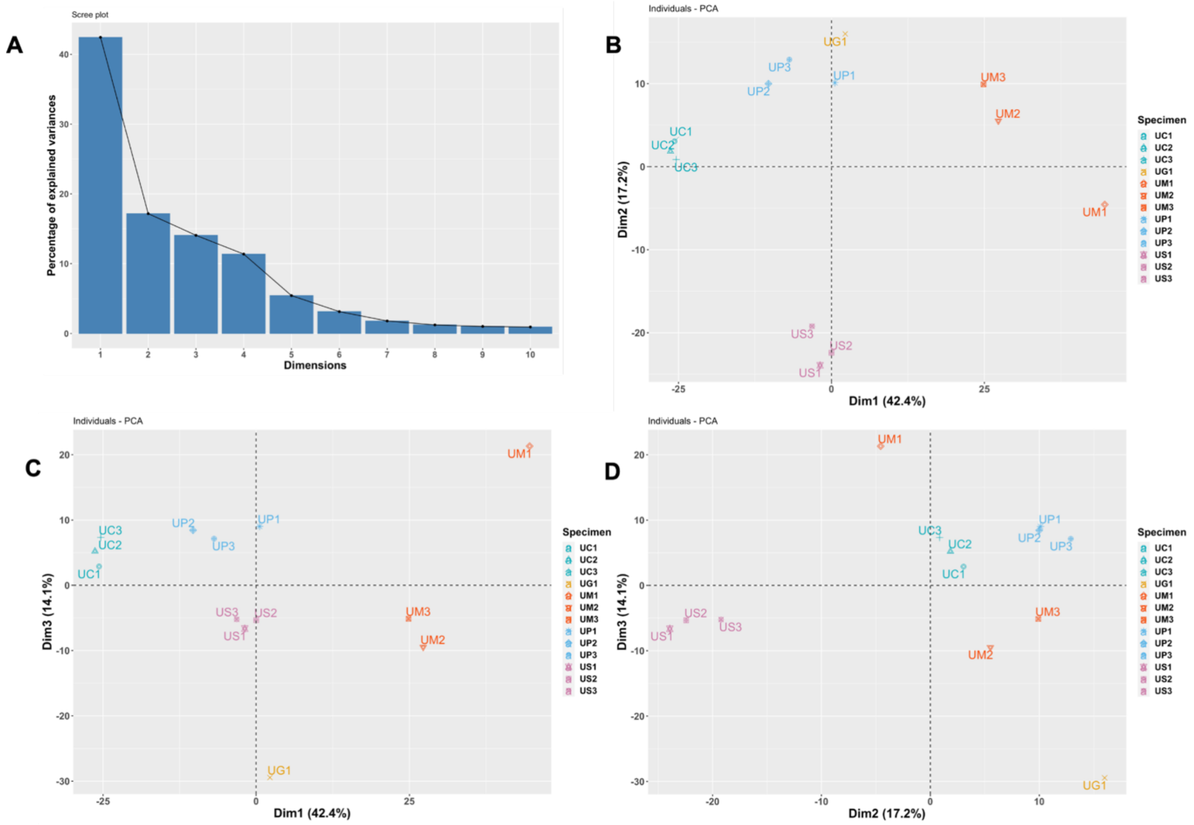


Fig. S4 Principal component analysis using the top 1’000 most variable transcripts. A. Scree plot of the components’ explained variance. **B, C, D**. PCA plot with the first two component (**B**), the first and third (**C**), and second and third components (**D**). UC1, UC2, UC3: chelicerae; UG1: gonad; UM1, UM2, UM3: midgut gland; UP1, UP2, UP3: prosoma; US1, US2, US3: silk glands.

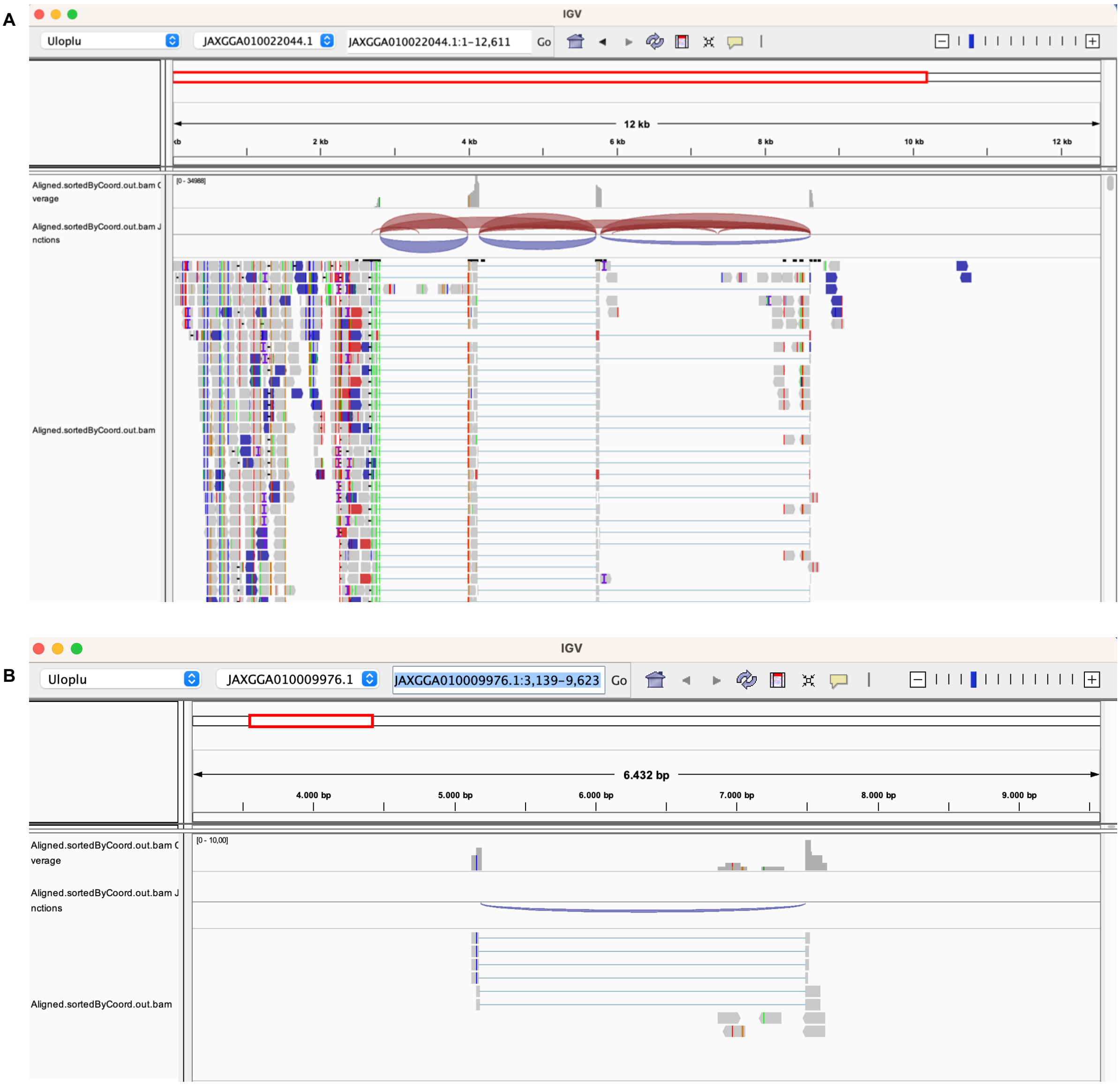


Fig. S5 Top defensin blast-hit regions of *U. plumipes* genome. A. Most RNA-Seq reads align to the top BlastN hit, which was 100% identical to our defensin transcript. **B.** Very few reads map to the second BlastN hit.

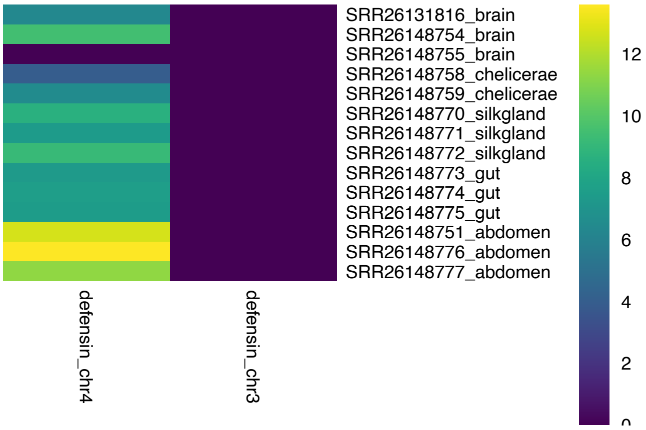
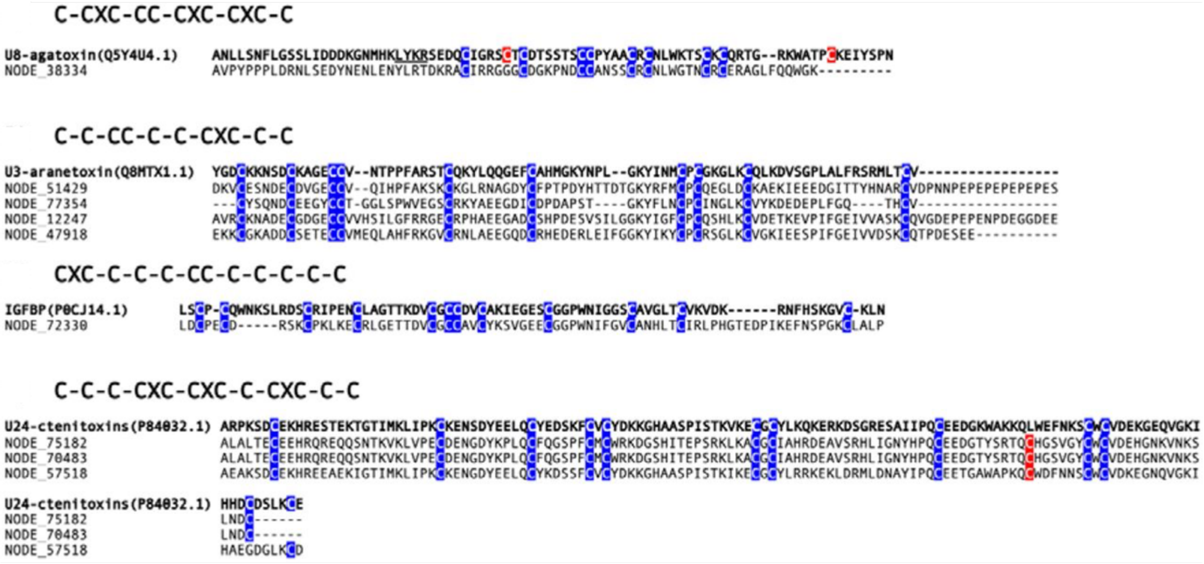


Fig. S6 Expression levels of the newly annotated defensin genes in the *Octonoba sinensis* genome. Expression levels as $\log_2$ (TPM).



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C-CXC-CC-CXC-CXC-C

U8-agatoxin(Q5Y4U4.1)      ANLLSNFLGSSLIDDDKGNMHKLYKRSEDQCIGRSCTCDTSSTSCCPYAACRCNLWKTSCKCQRTG--RKWATPCKEIYSPN
NODE_38334                 AVPYPPPLDRNLSEDYNENLENYLRTDKRACIRRGGGCDGKPNDCCANSSCRCNLWGTNCRCERAGLFQQWGK---------

C-C-CC-C-C-CXC-C-C

U3-aranetoxin(Q8MTX1.1)    YGDCKKNSDCKAGECCV--NTPPFARSTCQKYLQQGEFCAHMGKYNPL--GKYINMCPCGKGLKCQLKDVSGPLALFRSRMLTCV-----------------
NODE_51429                 DKVCESNDECDVGECCV--QIHPFAKSKCKGLRNAGDYCFPTPDYHTTDTGKYRFMCPCQEGLDCKAEKIEEEDGITTYHNARCVDPNNPEPEPEPEPEPEPES
NODE_77354                 ---CYSQNDCEEGYCCT-GGLSPWVEGSCRKYAEEGDICDPDAPST----GKYFLNCPCINGLKCVYKDEDEPLFGQ----THCV-----------------
NODE_12247                 AVRCKNADECGDGECCVVHSILGFRRGECRPHAEEGADCSHPDESVSILGGKYIGFCPCQSHLKCVDETKEVPIFGEIVVASKCQVGDEPEPENPDEGGDEE
NODE_47918                 EKKCGKADDCSETECCVMEQLAHFRKGVCRNLAEEGQDCRHEDERLEIFGGKYIKYCPCRSGLKCVGKIEESPIFGEIVVDSKCQTPDESEE----------

CXC-C-C-C-CC-C-C-C-C-C

IGFBP(P0CJ14.1)          LSCP-CQWNKSLRDSCRIPENCLAGTTKDVCGCCDVCAKIEGESCGGPWNIGGSCAVGLTCVKVDK------RNFHSKGVC-KLN
NODE_72330               LDCPECD-----RSKCPKLKECRLGETTDVCGCCAVCYKSVGEECGGPWNIFGVCANHLTCIRLPHGTEDPIKEFNSPGKCLALP

C-C-C-CXC-CXC-C-CXC-C-C

U24-ctenitoxins(P84032.1) ARPKSDCEKHRESTEKTGTIMKLIPKCKENSDYEELQCYEDSKFCVCYDKKGHAASPISTKVKECGCYLKQKERKDSGRESAIIPQCEEDGKWAKKQLWEFNKSCWCVDEKGEQVGKI
NODE_75182                ALALTECEEHRQREQQSNTKVKLVPECDENGDYKPLQCFQGSPFCMCWRKDGSHITEPSRKLKACGCIAHRDEAVSRHLIGNYHPQCEEDGTYSRTQCHGSVGYCWCVDEHGNKVNKS
NODE_70483                ALALTECEEHRQREQQSNTKVKLVPECDENGDYKPLQCFQGSPFCMCWRKDGSHITEPSRKLKACGCIAHRDEAVSRHLIGNYHPQCEEDGTYSRTQCHGSVGYCWCVDEHGNKVNKS
NODE_57518                AEAKSDCEKHREEAEKIGTIMKLIPKCKENGDYEELQCYKDSSFCVCYDKKGHAASPISTKIKECGCYLRRKEKLDRMLDNAYIPQCEETGAWAPKQCWDFNNSCWCVDKEGNQVGKI

U24-ctenitoxins(P84032.1) HHDCDSLKCE
NODE_75182                LNDC------
NODE_70483                LNDC------
NODE_57518                HAEGDGLKCD
```

Fig. S7 Alignment of predicted neurotoxins from the *U. plumipes* transcriptome. The cysteine frame is reported.