

Supplementary material for:

Associating biological activity and predicted structure of antimicrobial peptides from amphibians and insects

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Additional File 1

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Supplementary Tables

Table S1. Minimum inhibitory concentrations of peptides from three independent experiments

Peptide		MIC (μg/mL)		
		<i>E. coli</i> ATCC 25922	<i>Salmonella</i> Enteritidis	<i>S. aureus</i> ATCC 29213
GILDTLKQLGKAAVQGLLSKAACKLAKTC	AmMa1	2-4	16-32	8
DNKWQNVHFHRSVAVTGPTSFSFSHK	AnF11	>128	>128	>128
GILRSLGWIQMPRSRRRHR	AnF12	16-32	128	>128
GIYTGRLLPVYIPQPRPPHRLRR	ApCe1	4-8	16-32	>128
VKCRVRR	ApMe1	>128	>128	>128
GAHKEVFKRDTALTKEAAKKAKK	ApMe2	>128	>128	>128
GWGLINIKIPPVLHKVSVPLVSKR	ApMe3	>128	>128	>128
KHHHIKLRHERHRRYILKSLI	ApMe4	>128	>128	>128
SILSTLSHKR	ApMe5	>128	>128	>128
RARKIRRRRGSLRHCVTIPSTPSGR	ApMe6	>128	>128	>128
AAGAGKVTKSAQKAQKAK	BoAr1	>128	>128	>128
ATAAECLKHPWLKIKK	BoAr2	>128	>128	>128
IIRATAAECLKHPWLKIKK	BoAr3	>128	>128	>128
SVASLAKNSAWPVSLKR	BoAr4	>128	>128	>128
VTISIARRVSSHKRG	BoAr5	>128	>128	>128
GILRLVTRRRFRSPTNLNRYTVARLVSGVP	BoAr6	32	64	64-128
NKIKFINKYVKKVQLKKILVKS	BoCo1	>128	>128	>128
RKIIAVSVHKLRCRVKR	BoUs1	64-128	64	>128
FACPIGFFRLKR	CaCa1	128	>128	>128
FIKTQVLKHLVAGVRVARGLDWKWR	CaCa2	8	>128	64-128
KHHHIKLRHGRHRRSVLRTL	CaCa3	>128	>128	>128
RRFFATAPCGYSRKFKITRRKR	CaCa4	16-32	32-64	>128
GAFVLWGPTPRRRR	DiLo1	128	>128	>128
GIFSLIKGAAK	LeBo1	>128	>128	>128
GWLDIAKKVASVVAGIVKR	LiVe1	4-8	32	16-32
GWLDIAKKVASVVAGLGKR	LiVe2	4-8	64-128	64
VMLPKFKR	MiDe1	>128	>128	>128
RRAIFASIRGYLGRLKR	MyGu1	4	8-16	128
TPLSDIFRGQLRSRVSR	NaVi1	>128	>128	>128
SSLSPSSSSGLGKKKKRKSKRASR	NaVi2	>128	>128	>128

KLFLTLWKLKR	NaVi3	8-16	64	128
GSSSRSCRCIRLSRLSSKRT	NaVi4	>128	>128	>128
GLLSGILGAGKKIVCGFSGLC	OdMa1	8-16	128	64
GLISGILGAGKKVLCGLSGLC	OdMa10	16-32	>128	64-128
GFMDTAKNVAKNVAVTLLYNLKCKITKAC	OdMa12	4	64	64-128
GFMDTAKNVAKNVAVTLLDNLKCKITKAC	OdMa13	64-128	>128	>128
GLLRGILGAGKKIVCGLSGLC	OdMa2	4	8-16	4-8
GLLSGLLGAGKKIVCGLSGMC	OdMa3	8-16	64	32
GILSGLLGAGKKIVC	OdMa4	8-16	32	>128
GILSGLLGAGKKIVCGLSGLC	OdMa5	8-16	64-128	8-16
GLLSGVLGVGKKIVCGLSGLC	OdMa6	8-16	128	16-32
GLLSGVLGVGKKVLCGLSGLC	OdMa7	8-16	128	16-32
GLISGILGAGKK	OdMa8	>128	>128	>128
GLISGILGAGKKVLC	OdMa9	64-128	128	>128
GILSGLLGAGKKLACGLIGLC	OdTo1	8	32-64	8-16
GIFGGHLKVGKKIACGLSGLC	OdTo2	32	>128	>128
GIFGGLLKEGKKIACGLSGLC	OdTo3	32	>128	>128
KLMIPRKKRGIFGGLLKVGKKIACGLSGLC	OdTo4	8-16	16	16
RPRPQQVPPRPPHRLRR	PaVa1	2-4	4-8	>128
KYHHIKLRHGRHRTIH	PaVa2	>128	>128	>128
ITEPVGTKAPTFTSELRGGWLKKR	PaVa3	>128	>128	>128
WALRWKTR	PaVi1	>128	>128	>128
GLLGKVLGVGKKVLCGVTGLC	PeNi1	4	8-16	4
GLLDVTVGAAKNVAGILLNKLKCKVTGDC	PeNi10	8	64-128	16-32
GILDTLKGAAKNVAGVLLDKLKCKITGGC	PeNi11	8-16	128	64
GAPKGCWTKSYPPKPCSGKR	PeNi12	>128	>128	>128
KEERGAPKGCWTKSYPPKPCSGKR	PeNi13	>128	>128	>128
GLWTTIKEGVKNFSVGVLDKIRCKITGGC	PeNi14	4-8	32	16-32
FLPSSPWNEGTYVLKKLKS	PeNi15	>128	>128	>128
ATAWKVPPGLQPIRPIRPLCGNDKS	PeNi16	>128	>128	>128
RMIRPPGFSPFRVAPASSLKR	PeNi17	>128	>128	>128
RPRWSHRSRR	PeNi18	>128	>128	>128
GLLGKVLGVGKKVLCVVSGLC	PeNi2	4	16-32	4
GIFSLIKGAAKVVAKGLG	PeNi3	16-32	64-128	64-128
GLLGKVLGVGKKVLC	PeNi4	8-16	4-8	4-8
GLLGKVLGVGKKVLCGVTGRERCQ	PeNi5	32	64	>128
AGLQFPVGRIHRHLKTR	PeNi6	>128	>128	>128
VIPFVASVAAEMMHVYCAASKRCKN	PeNi7	128	>128	>128
GILLNTLKGAAKNVAGVLLDKLKCKITGGC	PeNi8	8	64	4-8

GLLGKILGVGKKVLCGVSGLC	PeNi9	2-4	8-16	4-8
VAAFAIIGCLCCRRPRR	PoRo1	>128	>128	>128
ISIKEALEHSFFHTVPRKWCKKH	PoSn1	64	>128	>128
TALKSLILKKLAKLNM	PoSn2	64-128	>128	>128
FLPVVAGLAACKVLPISIICAVTKKC	RaCa15	8-16	>128	4-8
GLLSGILGAGKK	RaOm1	>128	>128	>128
GILSGLLGAGKKIVCGLSGMC	RaOm2	64-128	>128	>128
GIFSLIKGAACKVVAKGLGK	RaOm3	8-16	32-64	64
GLLGKVLGVGKKVLCGVSGRC	RaOm4	4-8	32	64-128
AGYSRMIRPPGFSFPRVAPASSLKR	RaOm5	>128	>128	>128
GLVGKLVKGGLKLIGHVANG	RaSi1	8-16	>128	64-128
FPPPFGR	RaSi2	>128	>128	>128
EEQRFLPVVAGLAACKVLPISIICAVTKKC	RaSy2	64	>128	64-128
KIKIPWGKVKDFLVGGMKAVGKK	TeBi1	1-2	4-8	4-8
VPFGLKPR	TeRu1	>128	>128	>128
AFVRILCYCCPRRIKRR	TeRu2	64-128	>128	128
AVLSFVHKLFNLFLHVDTSKGKCRATLQ	TeRu3	>128	>128	>128
SWLSKSVKLVNKKNYTRLEKLAKKKLFNE	TeRu4	1	2-4	>128
FILHAKKTRSAK	VeSi1	>128	>128	>128

Table S2. Highest Scoring BLASTp matches of validated mature AMPs to NCBI non-redundant protein database

Peptide			BLASTp Results			
ID	Source Organism	Accession	Label	Organism	% Identity	% Query Coverage
AmMa1	<i>Amolops mantzorum</i>	ADM34231.1	palustrin-2GN3 antimicrobial peptide precursor	<i>Amolops granulosis</i>	93	100
AnFl2	<i>Anterhynchium flavomarginatum</i>	NA				
ApCe1	<i>Apis cerana</i>	NA				
BoAr6	<i>Bombus ardens</i>	NA				
BoUs1	<i>Bombus ussuriensis</i>	NA				
CaCa1	<i>Camponotus castaneus</i> , <i>Odontomachus monticola</i> , <i>Polistes rothneyi</i> , <i>Polistes snelleni</i> , <i>Sphecidae</i> sp. KJ-8906, <i>Vespa dybowskii</i>	XP_044595060.1	uncharacterized protein LOC123272390	<i>Cotesia glomerata</i>	100	100
CaCa2	<i>Camponotus castaneus</i> , <i>Odontomachus monticola</i> , <i>Temnothorax rugatulus</i>	XP_032682614.1	E3 ubiquitin-protein ligase HECTD1 isoform X1	<i>Odontomachus brunneus</i>	100	100
CaCa4	<i>Camponotus castaneus</i>	XP_012226981.1	PREDICTED: F-box/LRR-repeat protein 20 isoform X1	<i>Linepithema humile</i>	95	92
DiLo	<i>Diachasmimorpha longicaudata</i>	XP_008546965.1	PREDICTED: ubiquitin carboxyl-terminal hydrolase 30 homolog	<i>Microplitis demolitor</i>	93	100
LiVe1	<i>Litoria verreauxii</i>	NA				
LiVe2	<i>Litoria verreauxii</i>	NA				
MyGu1	<i>Myrmecia gulosa</i>	NA				
NaVi3	<i>Nasonia vitripennis</i> x <i>Nasonia giraulti</i> F1	NA				
OdMa1	<i>Odorrana margaretae</i>	AWH66435.1	nigrocin-HL precursor	<i>Hylarana latouchii</i>	95	100
OdMa2	<i>Odorrana margaretae</i>	ABG76398.1	nigrosin-OG15 antimicrobial peptide precursor	<i>Odorrana grahami</i>	95	100
OdMa3	<i>Odorrana margaretae</i>	AWH66435.1	nigrocin-HL precursor	<i>Hylarana latouchii</i>	90	100
OdMa4	<i>Odorrana margaretae</i>	NA				
OdMa5	<i>Odorrana margaretae</i>	ACA81697.1	lividin-7b precursor	<i>Odorrana livida</i>	100	100
OdMa6	<i>Odorrana margaretae</i>	ADP05878.1	nigrosin-RA3 peptide precursor	<i>Odorrana andersonii</i>	100	100
OdMa7	<i>Odorrana margaretae</i>	ABG76389.1	nigrosin-OG21 antimicrobial peptide precursor	<i>Odorrana grahami</i>	100	100
OdMa9	<i>Odorrana margaretae</i>	NA				
OdMa10	<i>Odorrana margaretae</i>	ADM34194.1	nigrosin-MG1 antimicrobial peptide precursor	<i>Odorrana margaretae</i>	100	100
OdMa12	<i>Odorrana margaretae</i>	ABG76517.1	odorrain-F2 antimicrobial peptide precursor	<i>Odorrana grahami</i>	97	100
OdMa13	<i>Odorrana margaretae</i>	ABG76517.1	odorrain-F2 antimicrobial peptide precursor	<i>Odorrana grahami</i>	100	100
OdTo1	<i>Odorrana tormota</i>	ADE48808.1	nigrosin-SHa antimicrobial protein	<i>Odorrana schmackeri</i>	81	100

OdTo2	<i>Odorrana tormota</i>	AIU99961.1	nigrosin-2EV4	<i>Odorrana exiliversabilis</i>	71	100
OdTo3	<i>Odorrana tormota</i>	AIU99958.1	nigrosin-2EV1	<i>Odorrana exiliversabilis</i>	76	100
OdTo4	<i>Odorrana tormota</i>	AIU99958.1	nigrosin-2EV1	<i>Odorrana exiliversabilis</i>	78	77
PaVa1	<i>Partula varia</i>	XP_024940855.1	apidaecins type 14-like isoform X2	<i>Cephus cinctus</i>	100	100
PeNi1	<i>Pelophylax nigromaculatus</i>	AEM68196.1	nigrocin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	95	100
PeNi2	<i>Pelophylax nigromaculatus</i>	AEM68196.1	nigrocin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	95	100
PeNi3	<i>Pelophylax nigromaculatus</i>	AEM68180.1	esculentin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	100	100
PeNi4	<i>Pelophylax nigromaculatus</i>	NA				
PeNi5	<i>Pelophylax nigromaculatus</i>	AEM68196.1	nigrocin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	95	79
PeNi7	<i>Bufo gargarizans, Leptobrachium boringii, Megophrys sangzhiensis, Polypedates megacephalus, Pelophylax nigromaculatus, Rhacophorus dennysi, Rana omeimontis</i>	AEM68212.1	nigrocin-6N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	100	100
PeNi8	<i>Bufo gargarizans, Megophrys sangzhiensis, Polypedates megacephalus, Pelophylax nigromaculatus, Rhacophorus dennysi, Rana omeimontis</i>	AGE10575.1	pelophylaxin-2	<i>Pelophylax nigromaculatus</i>	100	100
PeNi9	<i>Leptobrachium boringii, Megophrys sangzhiensis, Polypedates megacephalus, Pelophylax nigromaculatus, Rhacophorus dennysi, Rana omeimontis</i>	AEM68196.1	nigrocin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	95	100
PeNi10	<i>Leptobrachium boringii, Polypedates megacephalus, Pelophylax nigromaculatus, Rhacophorus dennysi, Rana omeimontis</i>	AEM68233.1	ranatuerin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	100	100
PeNi11	<i>Leptobrachium boringii, Polypedates megacephalus, Pelophylax nigromaculatus, Rhacophorus dennysi, Rana omeimontis</i>	AIU99901.1	pelophylaxin-1 antimicrobial peptide precursor	<i>Pelophylax hubeiensis</i>	100	100
PeNi14	<i>Bufo gargarizans, Polypedates megacephalus, Pelophylax nigromaculatus, Rana omeimontis</i>	AIU99897.1	palustrin-2HB1	<i>Pelophylax hubeiensis</i>	86	100
PoSn1	<i>Polistes snelleni</i>	EFN63611.1	Phosphorylase b kinase gamma catalytic chain, skeletal muscle isoform	<i>Camponotus floridanus</i>	93	65
PoSn2	<i>Polistes snelleni</i>	NA				
RaCa15	<i>Rana catesbeiana</i>	P82871.1	RccName: Full=Brevinin-1SY	<i>Lithobates sylvaticus</i>	100	100
RaOm2	<i>Rana omeimontis</i>	ADE48808.1	nigrosin-SHa antimicrobial protein	<i>Odorrana schmackeri</i>	90	100
RaOm3	<i>Rana omeimontis</i>	AEM68180.1	esculentin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	100	100

RaOm4	<i>Rana omeimontis</i>	AEM68196.1	nigrocin-2N protein precursor, partial	<i>Pelophylax nigromaculatus</i>	95	100
RaSi1	<i>Allobates femoralis</i> , <i>Pristimantis toftae</i> , <i>Ranitomeya sirensis</i>	NA				
RaSy2	<i>Rana sylvatica</i>	ABK91541.1	brevinin-1CHb precursor, partial	<i>Lithobates chiricahuensis</i>	78	96
TeBi1	<i>Tetramorium bicarinatum</i>	W8GNV3.1	Full=M-myrmecitoxin(01)-Tb1a; Short=M-MYRTX(01)-Tb1a	<i>Tetramorium bicarinatum</i>	100	100
TeRu2	<i>Temnothorax rugatulus</i>	XP_020294524.1	dopamine receptor 2 isoform X1	<i>Pseudomyrmex gracilis</i>	94	100
TeRu4	<i>Temnothorax rugatulus</i>	XP_024884948.1	uncharacterized protein LOC112463023	<i>Temnothorax curvispinosus</i>	97	100

Table S3. Range of selectivity indices of the 51 bioactive AMPs

AMP	Selectivity Index		
	<i>E. coli</i> ATCC 25922	<i>Salmonella</i> Enteritidis	<i>S. aureus</i> ATCC 29213
AmMa1	32 - >64	≥16	4 - >8
AnFl2	>4 - >8	N/O*	>1
ApCe1	>16 - >32	N/O	>4 - >8
BoAr6	>4	>1 - >2	>2
BoUs1	>1 - >2	N/O	>2
CaCa1	>1	N/O	N/O
CaCa2	>16	>1 - >2	N/O
CaCa4	>4 - >8	N/O	>2 - >4
DiLo1	>1	N/O	N/O
LiVe1	>16 - >32	>4 - >8	>4
LiVe2	>16 - >32	>2	>1 - >2
MyGu1	>32	>1	>8 - >16
NaVi3	>8 - >16	>1	>2
OdMa1	>8 - >16	>2 - >4	>1
OdMa2	32	16 - 32	8 - 16
OdMa3	>8 - >16	>4	>2
OdMa4	>8 - >16	N/O	>4
OdMa5	8 - 16	8 - 16	1 - 2
OdMa6	>8 - >16	>4 - >8	>1
OdMa7	>8 - >16	>4 - >8	>1
OdMa9	>1 - >2	N/O	>1
OdMa10	>4	>1 - >2	N/O
OdMa12	32	>1 - >2	>2
OdMa13	>1 - >2	N/O	N/O
OdTo1	≥16	8 - >16	2 - >4
OdTo2	>4	N/O	N/O
OdTo3	>2 - >4	N/O	N/O
OdTo4	>8 - >16	>8	>8
PaVa1	>32 - >64	N/O	>16 - >32
PeNi1	≥32	≥32	8 - >16
PeNi2	>32	>32	>4 - >8
PeNi3	>4 - >8	>1 - >2	>1 - >2
PeNi4	>8 - >16	>16 - >32	>16 - >32
PeNi5	>4	N/O	>2
PeNi7	>1	N/O	N/O
PeNi8	>16	>16 - >32	>2

PeNi9	32 - >64	16 - >32	8 - >16
PeNi10	>16	>4 - >8	>1 - >2
PeNi11	>8 - >16	>2 - >4	>1
PeNi14	>16 - >32	>4 - >8	>4
PoSn1	>2	N/O	N/O
PoSn2	>1 - >2	N/O	N/O
RaCa15	4 - 16	8 - 32	N/O
RaOm2	>1 - >2	N/O	N/O
RaOm3	>8 - >16	>2	>2 - >4
RaOm4	>16 - >32	>1 - >2	>4
RaSi1	>8 - >16	>1 - >2	N/O
RaSy2	>2	>1 - >2	N/O
TeBi1	>64 - >128	>16 - >32	>16 - >32
TeRu2	>1 - >2	N/O	N/O
TeRu4	>128	N/O	>32 - >64

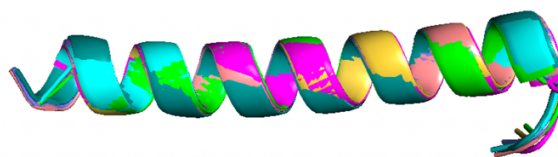
*N/O indicates no observed antimicrobial activity against the given bacterial species

Supplementary Figures

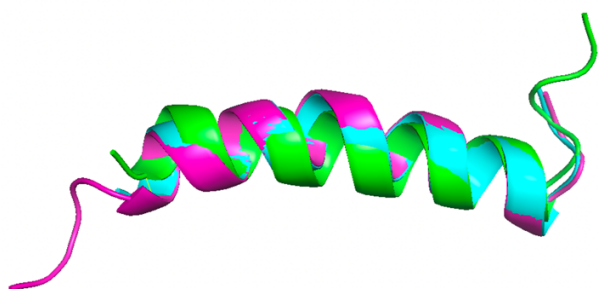
Apidaecin (2)



Brevinin-2 (7)



Brevinin-1 (3)



Nigrocin-2 (18)



Figure S1. Superimposed predicted 3D structures of AMP family members. Peptides identified as belonging to the Apidaecin, Brevinin-1, Brevinin-2 and Nigrocin-2 peptides within the 51 bioactive peptides are depicted.