Table S1. Genomic/transcriptomic evidence for the existence of GSEFLamides in the Arthropoda

Subphylum	Class (subclass)	Order	Example species (Accession No.)
Crustacea	Branchiopoda	Anostraca	None found
		Notostraca	*Triops cancriformis* (**BAYF01005563**); *Triops newberryi* (**GEHY01007259**)
		Laevicaudata	None found
		Spinicaudata	*Eulimnadia texana* (**NKDA01000001**)
		Cyclestherida	None found
		Cladocera	*Daphnia magna* (**LRGB01003056**); *Daphnia pulex* (**FE418902**; **FE418903**; **ACJG01004393**); *Diaphanosoma celebensis* (**GGQP01072647**)
	Remipedia		None found
	Cephalocarida		None found
	Maxillopoda (Copepoda)	Calanoida	*Calanus finmarchicus* (**GBFB01037216**)
		Cyclopoida	*Eucyclops serrulatus* (**GARW01034605**; **GARW01034606**); *Oithona nana* (**FTRT01000010**); *Paracyclopina nana* (**GCJT01011701**)
		Gelyelloida	None found
		Harpacticoida	*Tigriopus californicus* (**JW505134**; **JW524533**); *Tigriopus japonicas* (**GCHA01006330**); *Tisbe holothuriae* (**HAHV01208940**)
		Misophrioida	None found
		Monstrilloida	None found
		Mormonilloida	None found
		Platycopioida	None found
		Poecilostomatoida	None found
		Siphonostomatoida	*Caligus rogercresseyi* (**GAZX01021255**; **GAZX01021254**; **LBBU01194569**); *Lepeophtheirus salmonis* (**HACA01020923**; **LBBW01059486**); *Tracheliastes polycolpus* (**GGQW01002811**)
	Maxillopoda (Thecostraca)	Cirripedia	*Pollicipes pollicipes* (**GGCH01051771**)
		Facetotecta	None found
		Ascothoracida	None found
	Maxillopoda (Branchiura)		None found
	Maxillopoda (Pentastomida)		None found
	Maxillopoda (Mystacocarida)		None found
	Maxillopoda (Tantulocarida)		None found
	Ostracoda		None found
	Malacostraca (Eumalacostraca)	Bathynellacea	None found
		Anaspidacea	None found
		Spelaeogriphacea	None found
		Thermosbaenacea	None found
		Lophogastrida	None found
		Mysida	None found
		Mictacea	None found
		Amphipoda	*Hyalella azteca* (**XM_018163644**; **GEHV01118052**; **GEHV01118053**; **JQDR02014982**); *Parhyale hawaiensis* (**LQNS02278091**); *Talitrus saltator* (**GDUJ01078702**)
		Isopoda	*Proasellus arthrodilus* (**HAEQ01103713**); *Proasellus cantabricus* (**HAER01069123**); *Proasellus granadensis* (**HAEY01024833**); *Proasellus margalefi* (**HAFD01019418**); *Proasellus parvulus* (**HAFG01006587**); *Proasellus rectus* (**HAFI01120250**); *Ligia exotica* (**BDMT011533477**)
		Euphausiacea	*Euphausia superba* (**GFCS01030727**); *Meganyctiphanes norvegica* (**GETT01032249**)
		Decapoda	*Astacus astacus* (**GEDF01032051**; **GEDF01032046**); *Cancer borealis* (**GEFB01024463**); *Carcinus maenas* (**GFXF01018114**); *Caridina multidentata* (**BDMR012243044**); *Cherax quadricarinatus* (**MH210976**;

			HACK01049432; **HACK01072863**); *Eriocheir sinensis* (**LQIF01001265**; **GBZW01010051**; **GFBL01010056**); *Homarus americanus* (**GEBG01035690**; **GFDA01138849**; **GFDA01138850**; **GFDA01064392**; **MH615811**; **MH615812**; **MH615813**); *Jasus edwardsii* (**GGHM01077724**); *Litopenaeus vannamei* (**GFRP01041795**; **JP369300**; **JP405994**); *Marsupenaeus japonicus* (**CI999226**); *Palaemon varians* (**GFPG01041628**; **GFPG01046114**); *Penaeus monodon* (**NIUS011950493**); *Procambarus clarkii* (**GBEV01013249**; **GARH01032050**; **GARH01004165**); *Procambarus virginalis* (**MRZY010013654**); *Scylla paramamosain* (**KR078375**)
	Malacostraca (Hoplocarida)		None found
	Malacostraca (Phyllocarida)		None found
Hexapoda	Entognatha	Collembola	*Folsomia candida* (**XM_022110878**; **LNIX01000045**); *Holacanthella duospinosa* (**GFPE01084992**); *Orchesella cincta* (**LJIJ01000312**); *Pogonognathellus* sp. (**GATD02021554**); *Tetrodontophora bielanensis* (**GAXI02021309**)
		Diplura	*Catajapyx aquilonaris* (**JYFJ02003565**)
		Protura	*Acerentomon* sp. (**GAXE01002454**; **GAXE01002455**; **GAXE01016499**)
	Insecta	Zygentoma	*Atelura formicaria* (**GAYJ02039606**); *Thermobia domestica* (**GASN02037847**); *Tricholepidion gertschi* (**GASO02020345**)
		Ephemeroptera	*Baetis rhodani* (**LVVX01380025**); *Ephemera danica* (**AYNC02025439**); *Isonychia bicolor* (**GAXA02018803**)
		Odonata	*Calopteryx splendens* (**LYUA01002566**); *Ladona fulva* (**APVN02016086**); *Megaloprepus caerulatus* (**GEXY01535986**)
		Hemiptera	*Lygus hesperus* (**GBHO01039466**)
		Orthoptera	None found
		Mantodea	None found
		Blattodea	None found
		Dermaptera	None found
		Phasmatodea	None found
		Phthiraptera	None found
		Plecoptera	None found
		Grylloblattodea	None found
		Thysanoptera	None found
		Diptera	None found
		Hymenoptera	None found
		Lepidoptera	None found
		Mecoptera	None found
		Megaloptera	None found
		Neuroptera	None found
		Raphidioptera	None found
		Siphonaptera	None found
		Strepsiptera	None found
		Trichoptera	None found
Chelicerata	Arachnida	Acari	*Hypochthonius rufulus* (**GEYP01047459**; **LBFL01003433**); *Ixodes ricinus* (**JXMZ02002780**); *Ixodes scapularis* (**XM_002414888**; **EW84059**8; **PKSA01009712**); *Ornithodoros turicata* (**GDIE01026007**); *Platynothrus peltifer* (**GEYZ01012033**); *Rhipicephalus microplus* (**FG579568**); *Sarcoptes scabiei* (**BM522045**); *Steganacarus magnus* (**GEYQ01017742**; **LBFN01064525**); *Tetranychus urticae* (**XM_015935096**; **CAEY01000550**)
		Amblypygi	None found
		Araneae	*Latrodectus geometricus* (**GBJM01053139**); *Latrodectus hesperus* (**GBCS01014425**; **GFDB01000183**; **JJRX02014372**); *Loxosceles reclusa* (**JJRW010100076**); *Nephila clavipes* (**MWRG01026699**); *Parasteatoda tepidariorum* (**XR_001583087**; **AOMJ02232695**); *Pardosa pseudoannulata* (**GCKE01026805**); *Stegodyphus mimosarum* (**JT036531**; **AZAQ01120322**)
		Opiliones	None found
		Palpigradi	None found

		Pseudoscorpiones	*Cordylochernes scorpioides* (**QEEW01039327**)
		Ricinulei	None found
		Schizomida	None found
		Scorpiones	*Centruroides sculpturatus* (**XM_023362779**; **AXZI02005370**); *Mesobuthus martensii* (**AYEL01074582**); *Tityus serrulatus* (**GBZU01013855**)
		Solifugae	None found
		Thelyphonida	None found
	Pycnogonida		None found
	Merostomata		*Limulus polyphemus* (**XR_002609941**; **AZTN01025391**)
Myriapoda	Chilopoda		*Strigamia maritime* (**AFFK01019071**)
	Diplopoda		None found
	Pauropoda		None found
	Symphyla		*Symphylella vulgaris* (**GAKX01061676**)
Data from: Bao et al., 2015; Christie, 2014b, 2014d, 2014f, 2015a, 2015b, 2015c; Christie and Chi, 2015a, 2015b, 2015c; Christie and Pascual, 2016; Christie et al., 2015, 2017a, 2017b, 2018a, 2018b; Nguyen et al., 2016; Veenstra et al., 2012; this study.			

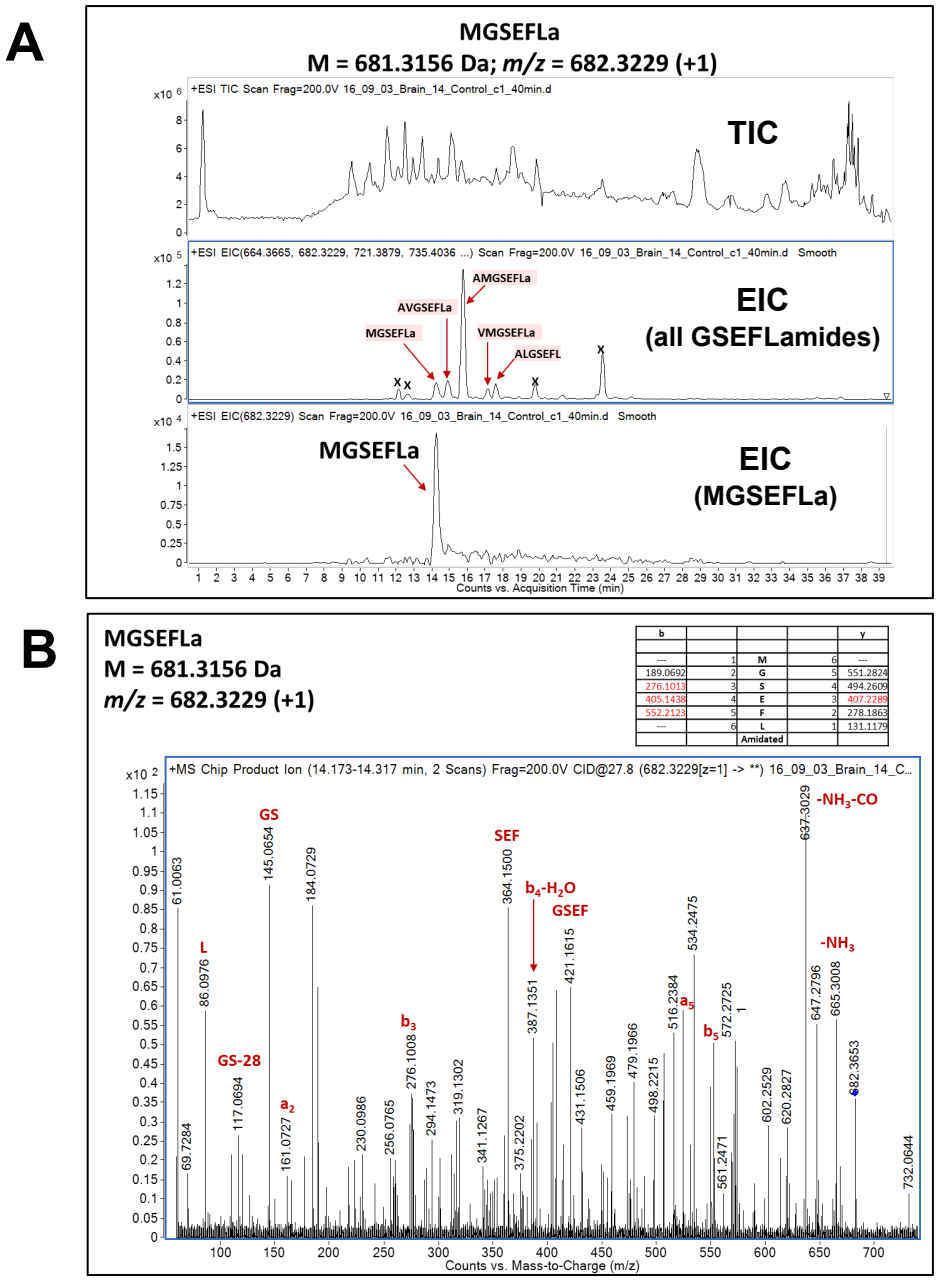
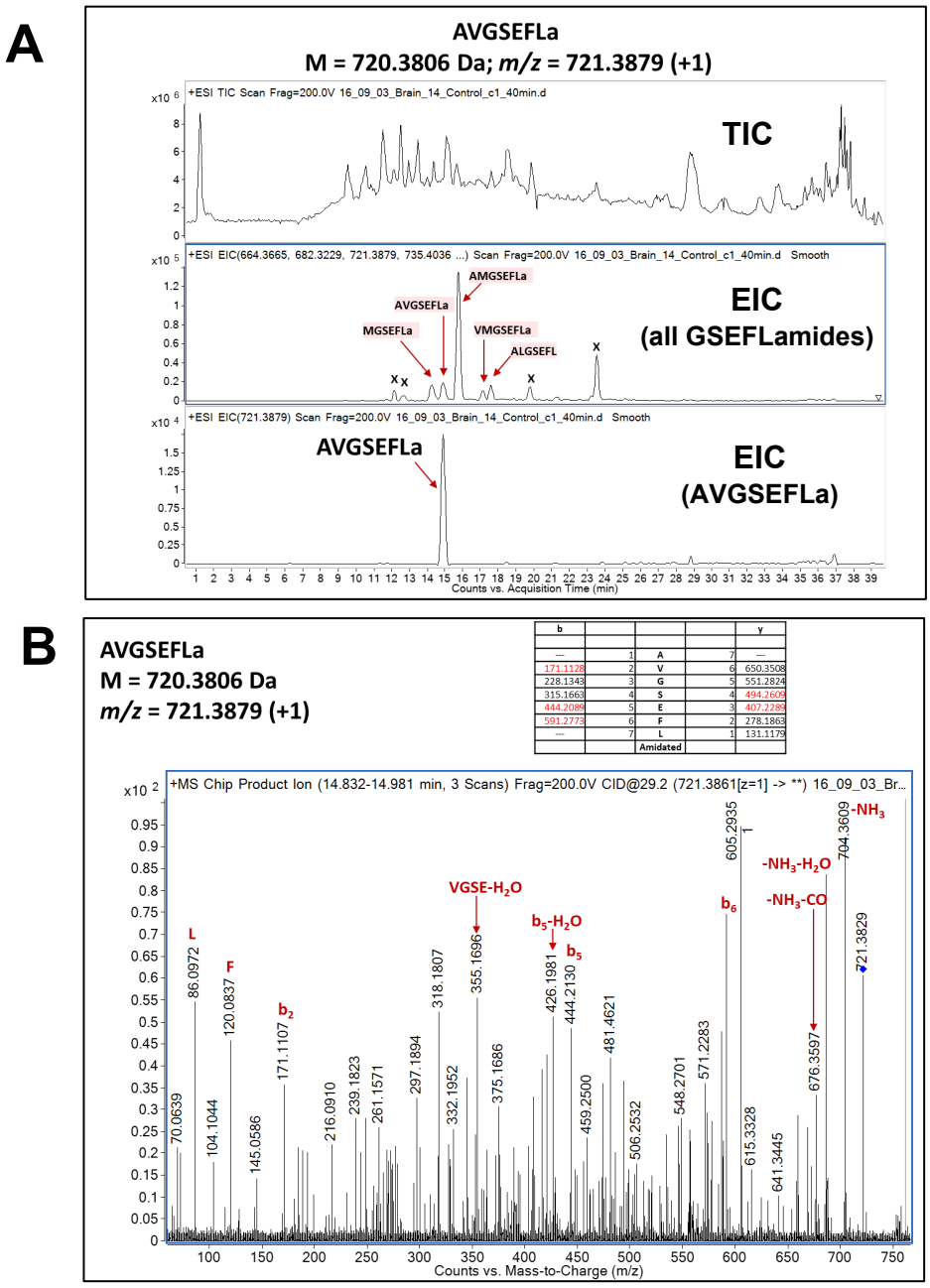
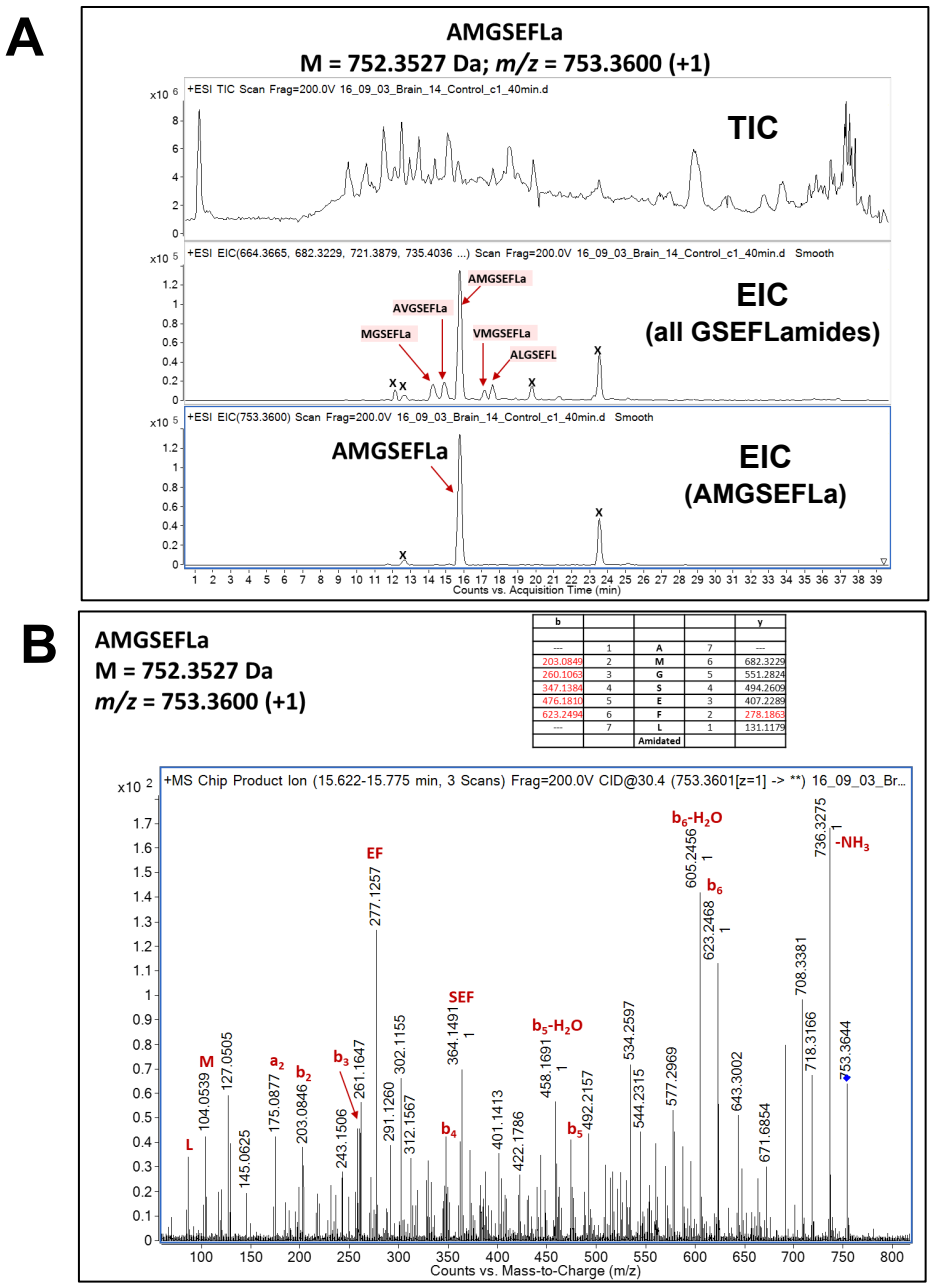


Figure S1. Chromatographic and mass spectrometric data in support of the identification of MGSEFLamide (A) Total ionization chromatogram (TIC) for *H. americanus* supraoesophageal ganglion (brain) extract, summed extracted ion chromatograms (EICs) for the $[M+H]^+$ peaks from IGSEFLa (*m/z* 664.3665), MGSEFLa, (*m/z* 682.3229), AVGSEFLa, (*m/z* 721.3880), AMGSEFLa, (*m/z* 753.3600), VMGSEFLa, (*m/z* 781.3914), and ALGSEFLa, (*m/z* 735.4036) and EIC for only MGSEFLa, (*m/z* 682.3229); "X" indicates that the signal does not originate from a GSEFLamide peptide; **(B)** MS/MS spectrum for the *m/z* 682.32, $[M+H]^+$ ion from MGSEFLa at a collision energy of 27.8 eV. The assigned sequence was supported by a complete series of N-terminus containing b-type product ions, many of which lost CO to produce a-type ions. C-terminus containing y-type ions provided additional sequence support, as did the detection of internal product ions, including GS, SEF, GSEF and immonium ions, including peaks for L and M. Monoisotopic masses are displayed.



b				y
---	1	A	7	---
171.1128	2	V	6	650.3508
228.1343	3	G	5	551.2824
315.1663	4	S	4	494.2609
444.2089	5	E	3	407.2289
591.2773	6	F	2	278.1863
---	7	L	1	131.1179
		Amidated		

Figure S2. Chromatographic and mass spectrometric data in support of the identification of AVGSEFLamide. (A) Total ionization chromatogram (TIC) for *H. americanus* supraoesophageal ganglion (brain) extract, summed extracted ion chromatograms (EICs) for the $[M+H]^+$ peaks from IGSEFLa (*m/z* 664.3665), MGSEFLa, (*m/z* 682.3229), AVGSEFLa, (*m/z* 721.3880), AMGSEFLa, (*m/z* 753.3600), VMGSEFLa, (*m/z* 781.3914), and ALGSEFLa, (*m/z* 735.4036) and EIC for only AVGSEFLa, (*m/z* 721.3880); "X" indicates that the signal does not originate from a GSEFLamide peptide; **(B)** MS/MS spectrum for the *m/z* 721.39, $[M+H]^+$ ion from AVGSEFLa at a collision energy of 29.2 eV. The assigned sequence was supported by a partial series of N-terminus containing b-type product ions, many of which lost CO to produce a-type ions. C-terminus containing y-type ions provided additional sequence support, as did the detection of internal product ions, including VGSE and immonium ions, including peaks for V, L and F. Monoisotopic masses are displayed.



b				y
---	1	A	7	---
203.0849	2	M	6	682.3229
260.1063	3	G	5	551.2824
347.1384	4	S	4	494.2609
476.1810	5	E	3	407.2289
623.2494	6	F	2	278.1863
---	7	L	1	131.1179
		Amidated		

Figure S3. Chromatographic and mass spectrometric data in support of the identification of AMGSEFLamide. (A) Total ionization chromatogram (TIC) for *H. americanus* supraoesophageal ganglion (brain) extract, summed extracted ion chromatograms (EICs) for the $[M+H]^+$ peaks from IGSEFLa (*m/z* 664.3665), MGSEFLa, (*m/z* 682.3229), AVGSEFLa, (*m/z* 721.3880), AMGSEFLa, (*m/z* 753.3600), VMGSEFLa, (*m/z* 781.3914), and ALGSEFLa, (*m/z* 735.4036) and EIC for only AMGSEFLa, (*m/z* 753.3600); "X" indicates that the signal does not originate from a GSEFLamide peptide; **(B)** MS/MS spectrum for the *m/z* 753.36, $[M+H]^+$ ion from AMGSEFLa at a collision energy of 30.4 eV. The assigned sequence was supported by a complete series of N-terminus containing b-type product ions, many of which lost CO to produce a-type ions. C-terminus containing y-type ions provided additional sequence support, as did the detection of internal product ions, including EF and SEF and immonium ions, including peaks for L, M and F. Monoisotopic masses are displayed.

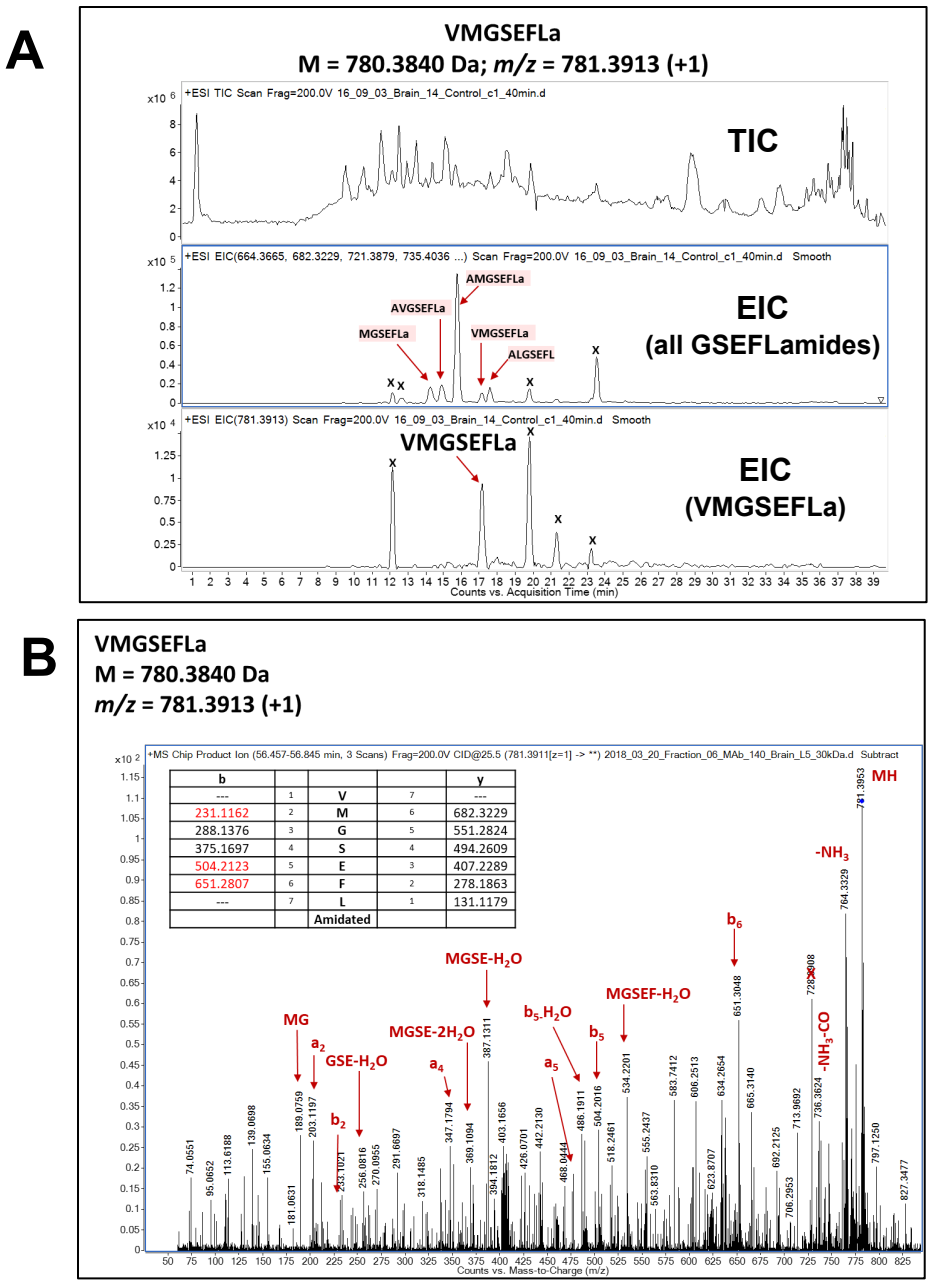


Figure S4. Chromatographic and mass spectrometric data in support of the identification of VMGSEFLamide. (A) Total ionization chromatogram (TIC) for *H. americanus* supraoesophageal ganglion (brain) extract, summed extracted ion chromatograms (EICs) for the $[M+H]^+$ peaks from IGSEFLa (*m/z* 664.3665), MGSEFLa, (*m/z* 682.3229), AVGSEFLa, (*m/z* 721.3880), AMGSEFLa, (*m/z* 753.3600), VMGSEFLa, (*m/z* 781.3914), and ALGSEFLa, (*m/z* 735.4036) and EIC for only VMGSEFLa, (*m/z* 781.3914); "X" indicates that the signal does not originate from a GSEFLamide peptide; **(B)** MS/MS spectrum for the *m/z* 781.39, $[M+H]^+$ ion from VMGSEFLa at a collision energy of 25.5 eV. The assigned sequence was supported by a partial series of N-terminus containing b-type product ions, many of which lost CO to produce a-type ions. The detection of internal product ions, including MS, GSE, and MGSE and immonium ions provided additional support. Monoisotopic masses are displayed.

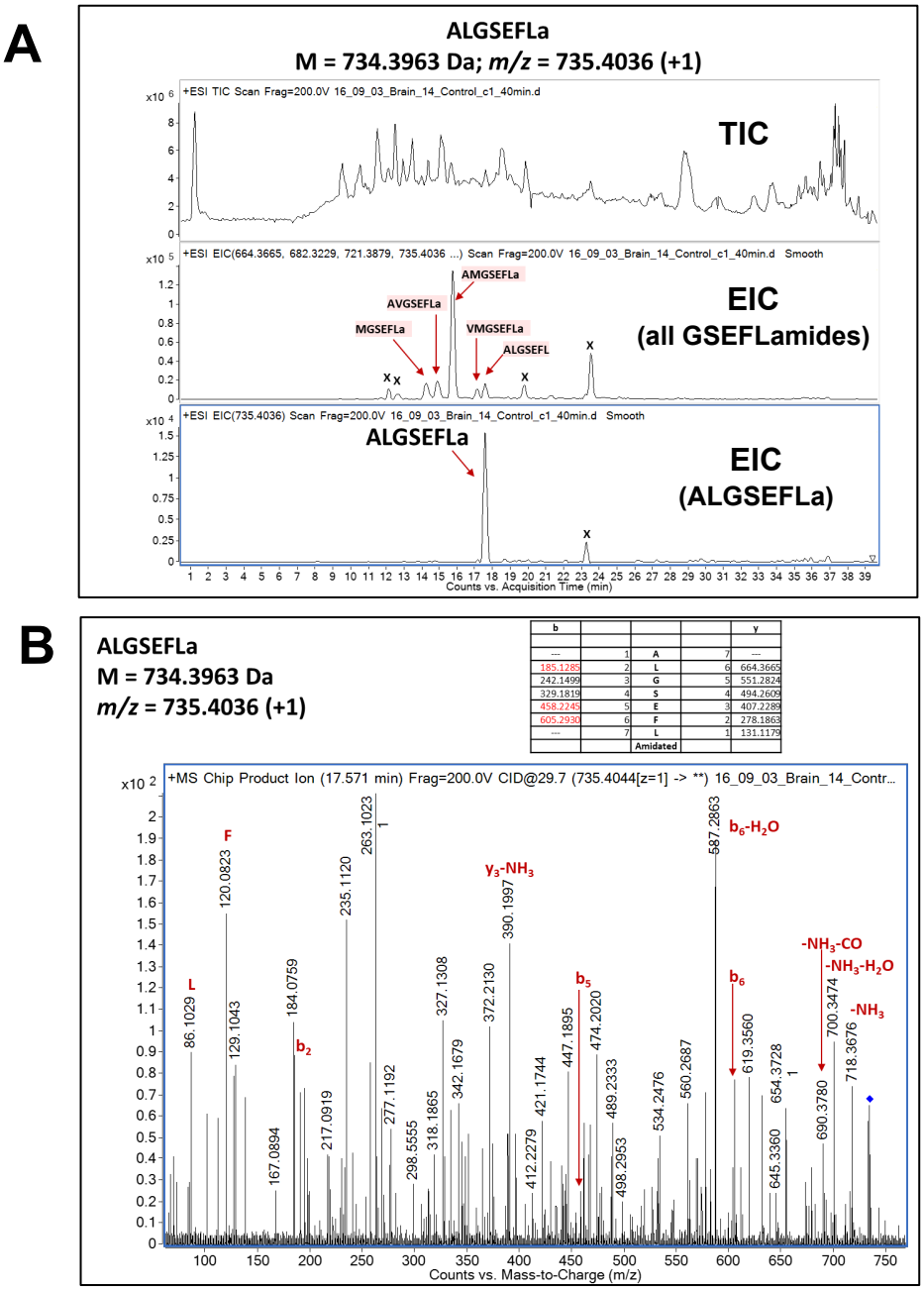


Figure S5. Chromatographic and mass spectrometric data in support of the identification of ALGSEFLamide. (A) Total ionization chromatogram (TIC) for *H. americanus* supraoesophageal ganglion (brain) extract, summed extracted ion chromatograms (EICs) for the $[M+H]^+$ peaks from IGSEFLa (*m/z* 664.3665), MGSEFLa, (*m/z* 682.3229), AVGSEFLa, (*m/z* 721.3880), AMGSEFLa, (*m/z* 753.3600), VMGSEFLa, (*m/z* 781.3914), and ALGSEFLa, (*m/z* 735.4036) and EIC for only ALGSEFLa, (*m/z* 735.4036); "X" indicates that the signal does not originate from a GSEFLamide peptide; **(B)** MS/MS spectrum for the *m/z* 735.40, $[M+H]^+$ ion from ALGSEFLa at a collision energy of 29.7 eV. The assigned sequence was supported by a partial series of N-terminus containing b-type product ions, many of which lost CO to produce a-type ions. C-terminus containing y-type ions provided additional sequence support, as did the detection of internal product ions and immonium ions, including peaks for L and F. Monoisotopic masses are displayed.