

Figure S1. Comparison of HPLC-MS chromatograms of the investigated *Chromodoris* species, mucus collected from the back (notum) and the trail of *C. annae*, and the associated sponge *Cacospongia mycofijiensis*, showing the presence of LatA (1) in all samples.

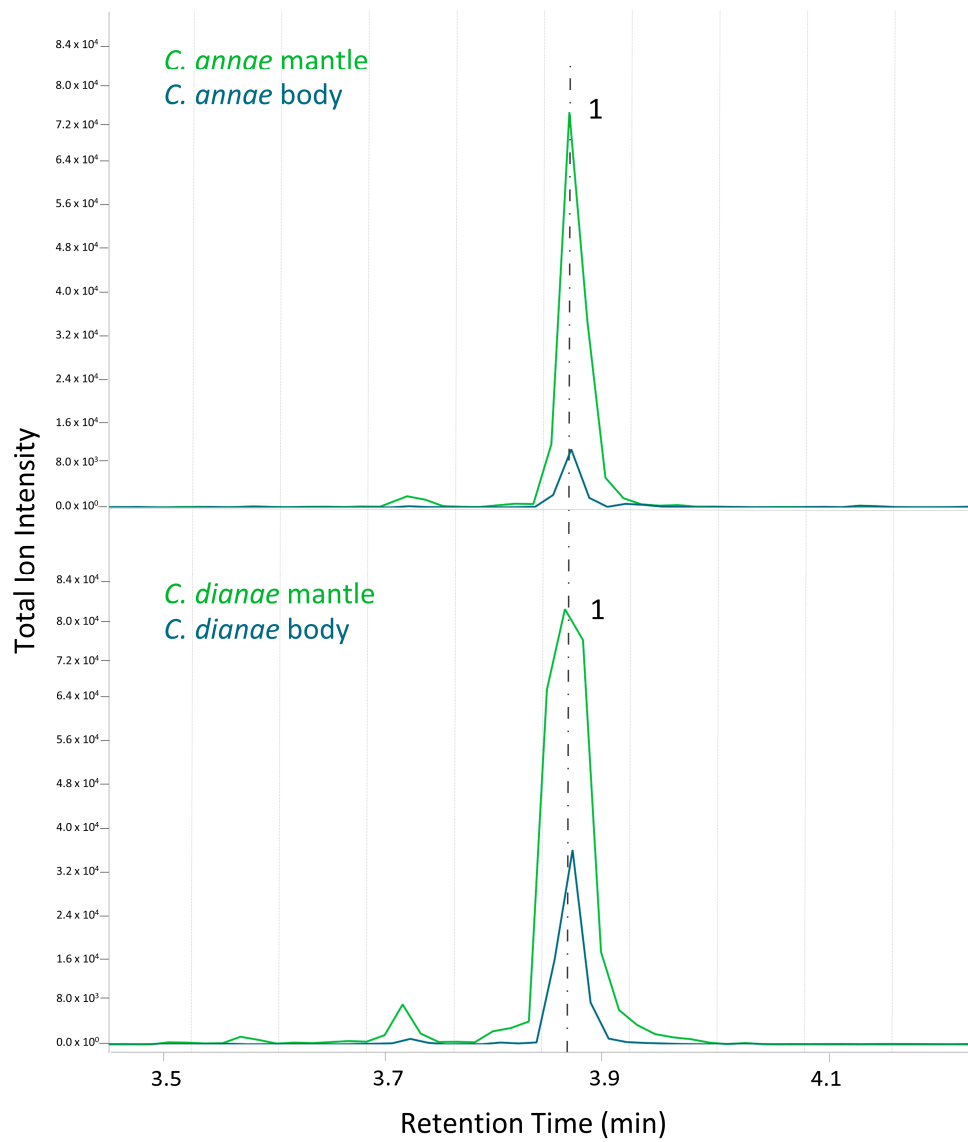


Figure S2. Comparison of superimposed HPLC–MS chromatograms of *C. annae* and *C. diana* mantle rim and body extracts, showing a high abundance of LatA (1) in the mantle rim.

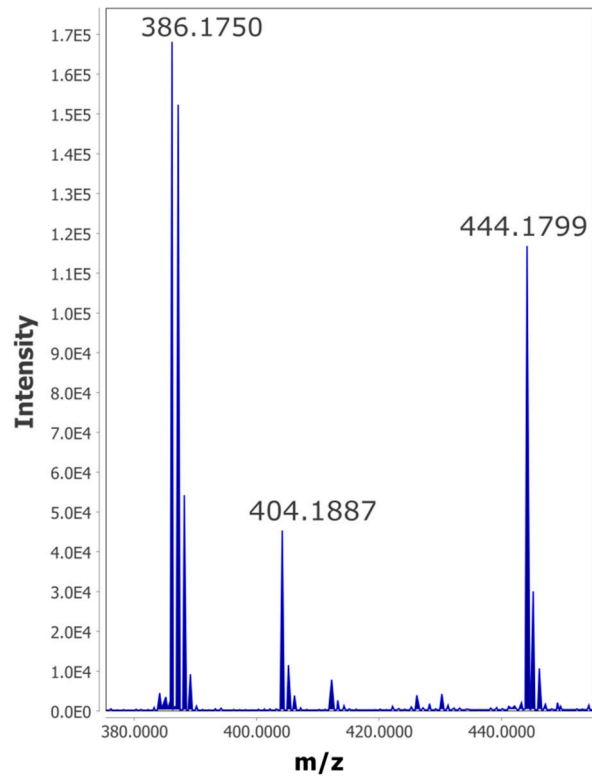


Figure S3. Exemplary mass spectrum from the chromatographic peaks marked as (1) in Figure S1 and Figure S2 showing characteristic protonated ion fragments and adducts for latrunculin A (m/z 386 $[M + H - 2H_2O]^+$, 404 $[M + H - H_2O]^+$ and 444 $[M + Na]^+$). Spectra were obtained in positive ion mode.

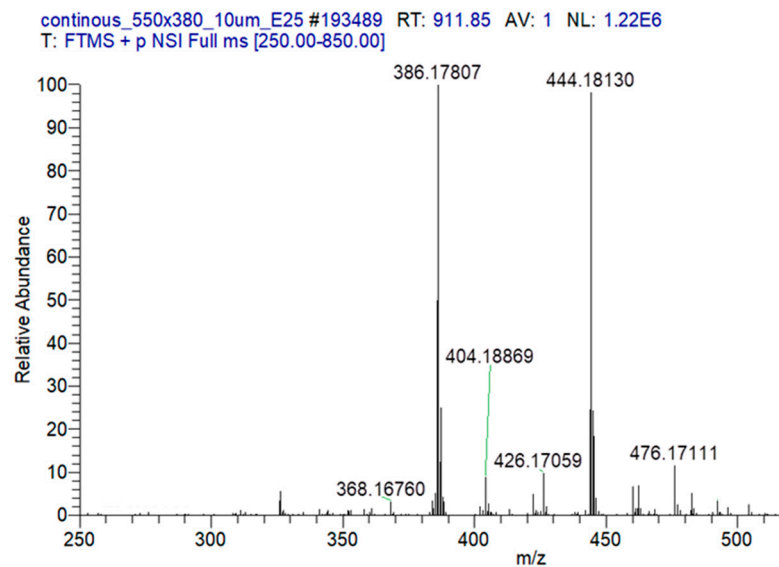


Figure S4. Single-pixel (10 μm) mass spectrum of the MALDI MSI from one of the *Chromodoris* MDF-vacuoles, showing protonated ion fragments and adducts typical for LatA (m/z 386 $[M + H - 2H_2O]^+$ and 444 $[M + Na]^+$) as main signals.

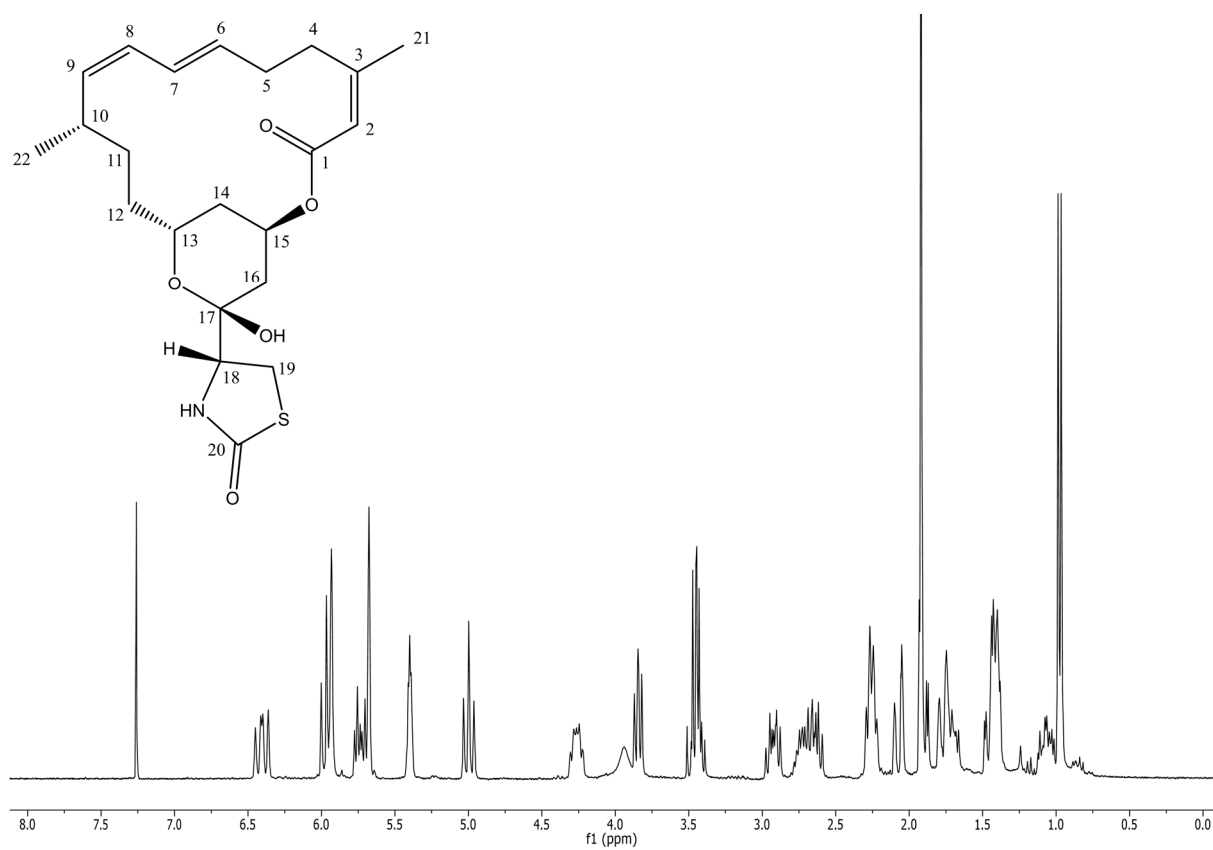


Figure S5. ^1H -NMR spectrum of latrunculin A in CDCl₃.

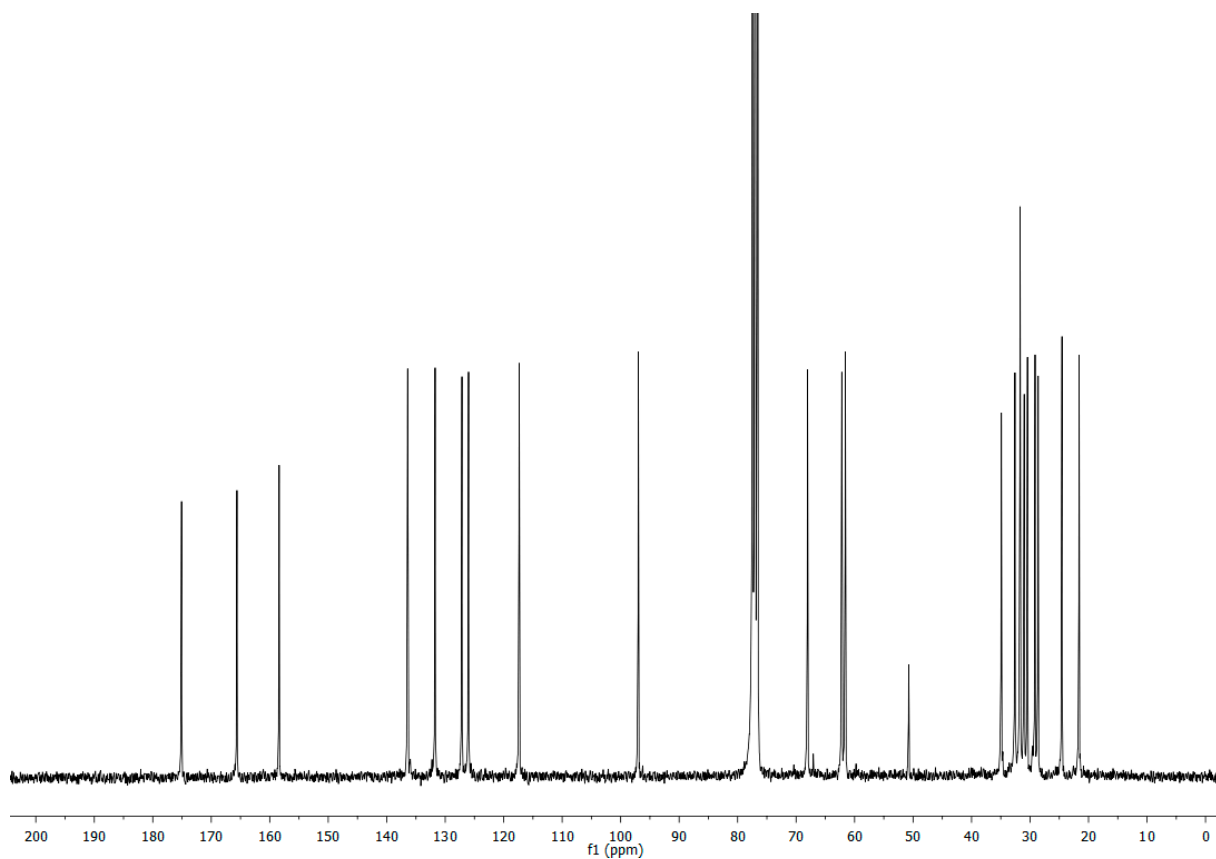


Figure S6. ^{13}C -NMR spectrum of latrunculin A in CDCl₃.

Table S1. A comparison of the ¹H-NMR Data reported for latrunculin A.

| C | Experimental Data (MeOD) δ _H | Experimental Data (CDCl ₃) δ _H | Kashman et al. 1980 [46] (CDCl ₃) δ _H | Groweiss et al., 1983 [112] (CDCl ₃) δ _H | Smith et al. 1992 [48] (CDCl ₃) δ _H | White et al. 1992 [147] (CDCl ₃) δ _H | Houssen et al. 2006 [38] (DMSO- <i>d</i> ₆) δ _H | Fürstner et al. 2007 [148] (CDCl ₃) δ _H |
|------|--|--|--|---|--|---|--|--|
| 1 | | | | | | | | |
| 2 | 5.62 brs | 5.68 brs | 5.69 d | 5.69 d | 5.62 s | 5.62 s | 5.54 d | 5.65–5.69 m |
| 3 | | | | | | | | |
| 4 α | 3.43–3.35 m | 3.48–3.39 m | | 3.00 dt | 3.39–3.43 m | | 3.18 dt | |
| 4 β | 2.52–2.34 m | 2.72 m | 2.60 m | 2.60 dt | 2.64–2.74 m | 2.74–2.63 m | 2.30 dt | 2.62–2.77 m |
| 5 α | 2.32 m | 2.66 m | 2.26 m | 2.26 m | 2.23–2.28 m | 2.30–2.26 m | 2.20 m | 2.23–2.34 m |
| 5 β | 2.24 m | 2.26 m | | 2.26 m | | | 2.12 m | |
| 6 | 5.76 ddd | 5.73 dt | 5.74 dt | 5.74 dt | 5.74 dt | 5.73 dt | 5.69 ddd | 5.74 s |
| 7 | 6.56 dd | 6.41 dd | 6.41 dd | 6.41 dd | 6.40 dd | 6.40 dd | 6.46 dd | 6.40 dt |
| 8 | 6.01 t | 5.97 t | 5.98 t | 5.98 t | 5.97 dd | 5.97 t | 5.93 | 5.97 dd |
| 9 | 4.98 m | 5.00 t | 5.02 t | 5.02 t | 5.01 dd | 5.01 t | 4.91 | 5.01 dd |
| 10 | 2.91 m | 2.92 m | 2.83 m | 2.83 m | 2.86–2.92 m | 2.91–2.85 m | 2.80 | 2.86–2.95 m |
| 11 α | 2.10 dt | 2.08 dt | | | 2.04–2.07 m | 2.06 dt | 1.69 | 2.04–2.07 m |
| 11 β | 1.79 dd | 1.75 m | | | | | 0.91 | |
| 12 | 1.08–1.02 m | 1.12–1.01 m | | | 1.06–1.11 m | 1.12–1.03 m | 1.22 | 1.01–1.14 m |
| 13 | 3.83 t | 3.85 t | 4.29 m | 4.29 m | 4.22–4.26 m | 4.25 m | 4.28 | 4.2–4.3 m |
| 14 α | 1.65 dd | 1.68 m | | | | 1.71 m | 1.42 bs | |
| 14 β | 1.48 m | 1.48 d | | | | 1.49–1.39 m | 1.32 ddd | |
| 15 | 5.17 brt | 5.40 p | 5.43 bt | 5.43 bt | 5.43 m | 5.43 p | 4.99 bs | 5.42 m |
| 16 | 1.41 dq | 1.42 m | | | 1.25–1.96 m | 1.82 d 1.79 d | 1.97 bd 1.53 dd | 1.24–1.98 m |
| 17 | | | | | | | | |
| 18 | 4.46 dt | 4.26 dt | 3.87 dd | 3.87 dd | 3.83–3.86 m | 3.89 s 3.85 dd | 3.63 dd | 3.82–3.93 m |
| 19 | 3.47 m | 3.45 m | 3.51 dd 3.48 dd | 3.51 dd | 3.46–3.51 m | 3.46 dd 3.44 dd | 3.48 dd 3.32 dd | 3.37–3.52 m |
| 20 | | | | | | | | |
| 21 | 1.94 s | 1.92 s | 1.92 d | 1.92 d | 1.93 s | 1.92 dd | 1.83 d | 1.93 s |
| 22 | 0.99 d | 0.98 d | 0.98 d | 0.98 d | 0.99 d | 0.98 d | 0.87 d | 0.98 d |
| NH | | | 6.3 | | | | 7.99 | |
| OH | | | 5.8 | 5.80 bs | 5.69 s | 5.69 s | 5.66 | 5.69 brs |

¹H (600 MHz), all δ in ppm relative to MeOD = 3.35/49.0, or CDCl₃ = 7.26/77.0.

Table S2. A comparison of the ¹³C-NMR Data reported for latrunculin A.

| C | Experimental Data (MeOD) δ _H | Experimental Data (CDCl ₃) δ _H | Kashman et al. 1980 [46] (CDCl ₃) δ _H | Groweiss et al., 1983 [112] (CDCl ₃) δ _H | Smith et al. 1992 [48] (CDCl ₃) δ _H | White et al. 1992 [147] (CDCl ₃) δ _H | Houssen et al. 2006 [38] (DMSO- <i>d</i> ₆) δ _H | Fürstner et al. 2007 [148] (CDCl ₃) δ _H | |
|----|---|---|--|---|--|---|--|--|-----------------|
| 1 | 168.5 | 165.6 | 166.0 | 166.0 | 165.3 | 165.3 | 166.3 | 165.4 | qC |
| 2 | 118.9 | 117.4 | 117.6 | 117.6 | 117.3 | 117.3 | 118.7 | 117.3 | CH |
| 3 | 160.1 | 158.4 | 158.3 | 158.3 | 158.5 | 158.4 | 157.8 | 158.3 | qC |
| 4 | 33.5 | 32.6 | 32.7 | 32.7 | 32.7 | 32.7 | 32.7 | 32.7 | CH ₂ |
| 5 | 31.8 | 30.4 | 30.6 | 30.6 | 30.4 | 30.4 | 30.8 | 30.5 | CH ₂ |
| 6 | 133.0 | 131.7 | 131.8 | 131.8 | 131.8 | 131.8 | 132.6 | 131.8 | CH |
| 7 | 127.6 | 126.0 | 126.3 | 126.3 | 126.0 | 126.0 | 126.8 | 126.0 | CH |
| 8 | 128.8 | 127.2 | 127.3 | 127.3 | 127.1 | 127.2 | 128.1 | 127.2 | CH |
| 9 | 137.1 | 136.4 | 136.5 | 136.5 | 136.5 | 136.5 | 136.5 | 136.5 | CH |
| 10 | 30.3 | 29.1 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 | CH |
| 11 | 32.1 | 31.7 | 31.2 | 31.2 | 31.0 | 31.0 | 31.6 | 31.0 | CH ₂ |
| 12 | 32.4 | 31.0 | 31.8 | 31.8 | 31.4 | 31.4 | 32.1 | 31.5 | CH ₂ |
| 13 | 64.5 | 62.2 | 62.3 | 62.3 | 62.3 | 62.3 | 61.6 | 62.3 | CH |
| 14 | 36.4 | 34.9 | 35.1 | 35.1 | 34.9 | 34.9 | 35.7 | 34.9 | CH ₂ |
| 15 | 69.3 | 68.1 | 68.1 | 68.1 | 68.2 | 68.2 | 67.6 | 68.2 | CH |
| 16 | 33.0 | 31.7 | 32.1 | 32.1 | 31.7 | 31.8 | 32.7 | 31.8 | CH ₂ |
| 17 | 97.9 | 97.0 | 96.9 | 96.9 | 97.3 | 97.3 | 96.4 | 97.3 | qC |
| 18 | 63.2 | 61.6 | 62.1 | 62.1 | 62.3 | 62.3 | 62.9 | 62.4 | CH |
| 19 | 29.6 | 28.6 | 28.7 | 28.7 | 28.7 | 28.7 | 28.5 | 28.7 | CH ₂ |
| 20 | 177.8 | 175.1 | 175.5 | 175.5 | 174.6 | 174.6 | 173.9 | 174.8 | qC |
| 21 | 25.0 | 24.5 | 24.7 | 24.7 | 24.5 | 24.5 | 25.0 | 24.5 | CH ₃ |
| 22 | 22.3 | 21.6 | 21.8 | 21.8 | 21.6 | 21.6 | 22.6 | 21.6 | CH ₃ |

¹³C NMR (150 MHz), all δ in ppm relative to MeOD = 3.35/49.0, or CDCl₃ = 7.26/77.0. Multiplicities determined by DEPT.

Latrunculin A: C₂₂H₃₁NO₅S, white amorphous solid (22.4 mg, ~400 µg/specimen); [α]_D²⁰ + 273.5 (c 0.1, CHCl₃); ¹H- and ¹³C-NMR (Tables S1 and S2.); HR-ESI-MS m/z 444.0638 [M + Na]⁺ (calcd. for C₂₂H₃₁NO₅S + Na, 444.1821).

Table S3. Amino acid mutations of actin proteins reported to inhibit binding of latrunculins.

| Amino Acid Mutations | Subdomain | References |
|----------------------|-----------|-----------------|
| Tyr 69 (Y) | II | [55,104] |
| Glu 117 (E) | III | [103] |
| Asp 157 (D) | III | [52,55,104] |
| Val 159 (V) | III | [99] |
| Arg 183 (R) | IV | [51,60,100,104] |
| Asp 184 (D) | IV | [51,60,104] |
| Thr 186 (T) | IV | [55,104] |
| Asp 187 (D) | IV | [101] |
| Arg 196 (R) | IV | [102] |
| Arg 210 (R) | IV | [51,55,104] |
| Asp 211 (D) | IV | [51] |
| Lys 213 (K) | IV | [51] |
| Glu 214 (E) | IV | [51,55,104] |
| Lys 215 (K) | IV | [51] |
| Arg 335 (R) | I | [105] |



Figure S7. *Chromodoris* specimens as found in the field on their substrates. (A) *C. diana* specimens found on *C. mycofijiensis* with visible feeding traces, left, and on a sponge belonging to the subfamily Thorectinae, right; (B) *C. annae* specimens found on *C. mycofijiensis* with feeding traces, left and middle; (C) *C. lochi* on a sponge belonging to the family Petrosiidae.

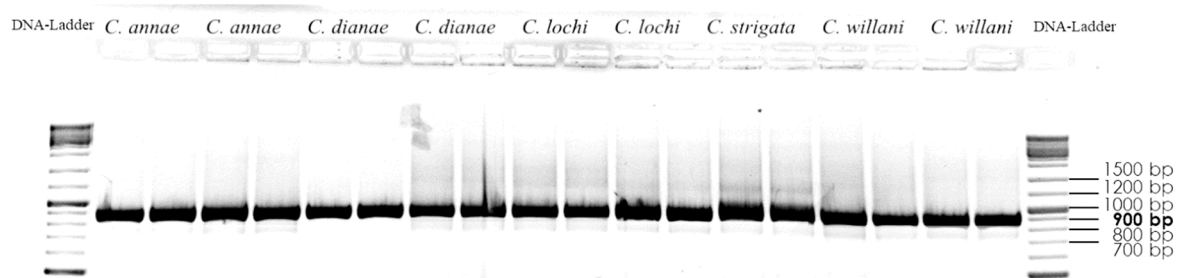


Figure S8. Exemplary gel-electrophoresis image of *Chromodoris* putative actin gene fragments (~885 bp), amplified by polymerase chain reaction (PCR) and stained with ethidium bromide.

Table S4. Recipe for 10 mL isotonic solution (~ 1000 mOsmoles).

| H ₂ Odest | 10 mL |
|--------------------------------------|----------|
| NaCl | 125.0 mg |
| Glucose | 62.4 mg |
| MgSO ₄ ·7H ₂ O | 62.4 mg |
| KCl | 3.44 mg |
| NaHCO ₃ | 1.92 mg |
| MgCl ₂ ·6H ₂ O | 57.0 mg |
| CaCl ₂ | 14.88 mg |

Table S5. NCBI BLAST result for *Chromodoris annae* actin isoform nucleotide sequence.

| Description | Scientific Name | Query Cover | Per. Ident | Accession |
|--|-----------------------------------|-------------|------------|----------------|
| PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA | <i>Urocitellus parryii</i> | 99% | 72.4% | XM_026412460.1 |
| PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA | <i>Urocitellus parryii</i> | 99% | 72.4% | XM_026412451.1 |
| PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA | <i>Ictidomys tridecemlineatus</i> | 99% | 72.1% | XM_005320387.2 |
| PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA | <i>Ictidomys tridecemlineatus</i> | 99% | 72.1% | XM_005320386.2 |
| PREDICTED: Pantherophis guttatus actin, gamma-enteric smooth muscle (LOC117673363), mRNA | <i>Pantherophis guttatus</i> | 99% | 71.9% | XM_034430717.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-16 | <i>Artemia franciscana</i> | 99% | 71.7% | AJ269582.1 |
| Dictyocaulus viviparus actin variant 2 mRNA, complete cds | <i>Dictyocaulus viviparus</i> | 99% | 71.6% | EU169822.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-14 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269586.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-3 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269583.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-13 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269580.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-9 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269579.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-11 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269578.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-18 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269581.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-19 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269577.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-15 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269575.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-17 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269574.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-1 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269567.1 |
| Artemia mRNA for actin (clone pArAct302) | <i>Artemia sp.</i> | 99% | 71.4% | X52604.1 |
| PREDICTED: Drosophila suzukii actin-87E (LOC118877550), mRNA | <i>Drosophila suzukii</i> | 99% | 71.4% | XM_036816658.1 |
| PREDICTED: Lepidothrix coronata actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Lepidothrix coronata</i> | 99% | 71.4% | XM_017803871.1 |
| PREDICTED: Chiroxiphia lanceolata actin alpha 2, smooth muscle (ACTA2), mRNA | <i>Chiroxiphia lanceolata</i> | 99% | 71.4% | XM_032695678.1 |
| PREDICTED: Corapipo altera actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Corapipo altera</i> | 99% | 71.2% | XM_027648332.1 |
| PREDICTED: Denticeps clupeoides actin, alpha skeletal muscle 2 (LOC114795339), mRNA | <i>Denticeps clupeoides</i> | 99% | 71.2% | XM_028988485.1 |
| PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X2, mRNA | <i>Erinaceus europaeus</i> | 99% | 71.1% | XM_007538841.2 |
| PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X1, mRNA | <i>Erinaceus europaeus</i> | 99% | 71.1% | XM_007538840.1 |
| PREDICTED: Chinchilla lanigera actin, alpha 1, skeletal muscle (Acta1), mRNA | <i>Chinchilla lanigera</i> | 99% | 71.1% | XM_005405008.1 |
| PREDICTED: Opisthocomus hoazin actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Opisthocomus hoazin</i> | 99% | 71.0% | XM_009935662.1 |

| | | | | |
|---|--|------------|--------------|-----------------------|
| PREDICTED: <i>Opisthocomus hoazin</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Opisthocomus hoazin</i> | 99% | 71.0% | XM_009935660.1 |
| PREDICTED: <i>Pan troglodytes</i> actin, alpha 1, skeletal muscle (ACTA1), mRNA | <i>Pan troglodytes</i> | 99% | 71.0% | XM_016940594.1 |
| PREDICTED: <i>Pterocles gutturalis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Pterocles gutturalis</i> | 99% | 70.9% | XM_010082746.1 |
| PREDICTED: <i>Pterocles gutturalis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Pterocles gutturalis</i> | 99% | 70.9% | XM_010082745.1 |
| PREDICTED: <i>Catharus ustulatus</i> actin alpha 2, smooth muscle (ACTA2), mRNA | <i>Catharus ustulatus</i> | 99% | 70.9% | XM_033065747.1 |
| PREDICTED: <i>Aptenodytes forsteri</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Aptenodytes forsteri</i> | 99% | 70.9% | XM_009277749.2 |
| PREDICTED: <i>Pygoscelis adeliae</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Pygoscelis adeliae</i> | 99% | 70.8% | XM_009320191.1 |
| PREDICTED: <i>Balearica regulorum gibbericeps</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Balearica regulorum gibbericeps</i> | 99% | 70.7% | XM_010305821.1 |
| PREDICTED: <i>Balearica regulorum gibbericeps</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Balearica regulorum gibbericeps</i> | 99% | 70.7% | XM_010305820.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X3, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897268.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X2, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897267.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X1, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897266.1 |
| PREDICTED: <i>Perca fluviatilis</i> actin, alpha cardiac muscle 1 (LOC120549587), mRNA | <i>Perca fluviatilis</i> | 99% | 70.7% | XM_039786604.1 |
| PREDICTED: <i>Orbicella faveolata</i> actin, cytoplasmic 1 (LOC110065202), mRNA | <i>Orbicella faveolata</i> | 99% | 70.6% | XM_020772311.1 |
| PREDICTED: <i>Fulmarus glacialis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Fulmarus glacialis</i> | 99% | 70.6% | XM_009576330.1 |
| PREDICTED: <i>Fulmarus glacialis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Fulmarus glacialis</i> | 99% | 70.6% | XM_009576329.1 |
| PREDICTED: <i>Gymnodraco acuticeps</i> actin, alpha skeletal muscle (LOC117544084), mRNA | <i>Gymnodraco acuticeps</i> | 99% | 70.5% | XM_034213253.1 |
| PREDICTED: <i>Hipposideros armiger</i> actin, alpha 1, skeletal muscle (ACTA1), transcript variant X2, mRNA | <i>Hipposideros armiger</i> | 99% | 70.5% | XM_019626360.1 |
| PREDICTED: <i>Hipposideros armiger</i> actin, alpha 1, skeletal muscle (ACTA1), transcript variant X1, mRNA | <i>Hipposideros armiger</i> | 99% | 70.5% | XM_019626358.1 |
| PREDICTED: <i>Sturnus vulgaris</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Sturnus vulgaris</i> | 99% | 70.4% | XM_014875386.1 |
| PREDICTED: <i>Pseudochaenichthys georgianus</i> actin, alpha skeletal muscle (LOC117468060), mRNA | <i>Pseudochaenichthys georgianus</i> | 99% | 70.4% | XM_034112020.1 |
| PREDICTED: <i>Trematomus bernacchii</i> actin, alpha skeletal muscle (LOC117478450), mRNA | <i>Trematomus bernacchii</i> | 99% | 70.4% | XM_034125513.1 |
| PREDICTED: <i>Vombatus ursinus</i> actin, alpha skeletal muscle (LOC114049606), mRNA | <i>Vombatus ursinus</i> | 99% | 70.4% | XM_027870867.1 |
| PREDICTED: <i>Vombatus ursinus</i> actin, alpha skeletal muscle (LOC114049602), mRNA | <i>Vombatus ursinus</i> | 99% | 70.4% | XM_027870861.1 |
| PREDICTED: <i>Phascolarctos cinereus</i> actin, alpha 1, skeletal muscle (ACTA1), mRNA | <i>Phascolarctos cinereus</i> | 99% | 70.3% | XM_021000483.1 |
| PREDICTED: <i>Charadrius vociferus</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Charadrius vociferus</i> | 99% | 70.3% | XM_009888704.1 |
| PREDICTED: <i>Charadrius vociferus</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Charadrius vociferus</i> | 99% | 70.3% | XM_009888702.1 |
| <u><i>Clupea harengus</i> actin alpha 1, skeletal muscle a (acta1a), mRNA</u> | <u><i>Clupea harengus</i></u> | <u>99%</u> | <u>70.3%</u> | <u>NM_001309834.1</u> |

Table S6. NCBI BLAST result for *Chromodoris annae* actin isoform nucleotide sequence.

| Description | Scientific Name | Query Cover | Per. Ident | Accession |
|--|-----------------------------------|-------------|------------|----------------|
| PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA | <i>Urocitellus parryii</i> | 99% | 72.4% | XM_026412460.1 |
| PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA | <i>Urocitellus parryii</i> | 99% | 72.4% | XM_026412451.1 |
| PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA | <i>Ictidomys tridecemlineatus</i> | 99% | 72.1% | XM_005320387.2 |
| PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA | <i>Ictidomys tridecemlineatus</i> | 99% | 72.1% | XM_005320386.2 |
| PREDICTED: Pantherophis guttatus actin, gamma-enteric smooth muscle (LOC117673363), mRNA | <i>Pantherophis guttatus</i> | 99% | 71.9% | XM_034430717.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-16 | <i>Artemia franciscana</i> | 99% | 71.7% | AJ269582.1 |
| Dictyocaulus viviparus actin variant 2 mRNA, complete cds | <i>Dictyocaulus viviparus</i> | 99% | 71.6% | EU169822.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-14 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269586.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-3 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269583.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-13 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269580.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-9 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269579.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-11 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269578.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-18 | <i>Artemia franciscana</i> | 99% | 71.5% | AJ269581.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-19 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269577.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-15 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269575.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-17 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269574.1 |
| Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-1 | <i>Artemia franciscana</i> | 99% | 71.4% | AJ269567.1 |
| Artemia mRNA for actin (clone pArAct302) | <i>Artemia sp.</i> | 99% | 71.4% | X52604.1 |
| PREDICTED: Drosophila suzukii actin-87E (LOC118877550), mRNA | <i>Drosophila suzukii</i> | 99% | 71.4% | XM_036816658.1 |
| PREDICTED: Lepidothrix coronata actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Lepidothrix coronata</i> | 99% | 71.4% | XM_017803871.1 |
| PREDICTED: Chiroxiphia lanceolata actin alpha 2, smooth muscle (ACTA2), mRNA | <i>Chiroxiphia lanceolata</i> | 99% | 71.4% | XM_032695678.1 |
| PREDICTED: Corapipo altera actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Corapipo altera</i> | 99% | 71.2% | XM_027648332.1 |
| PREDICTED: Denticeps clupeoides actin, alpha skeletal muscle 2 (LOC114795339), mRNA | <i>Denticeps clupeoides</i> | 99% | 71.2% | XM_028988485.1 |
| PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X2, mRNA | <i>Erinaceus europaeus</i> | 99% | 71.1% | XM_007538841.2 |
| PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X1, mRNA | <i>Erinaceus europaeus</i> | 99% | 71.1% | XM_007538840.1 |
| PREDICTED: Chinchilla lanigera actin, alpha 1, skeletal muscle (Acta1), mRNA | <i>Chinchilla lanigera</i> | 99% | 71.1% | XM_005405008.1 |
| PREDICTED: Opisthocomus hoazin actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Opisthocomus hoazin</i> | 99% | 71.0% | XM_009935662.1 |

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|---|--|------------|--------------|-----------------------|
| PREDICTED: <i>Opisthocomus hoazin</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Opisthocomus hoazin</i> | 99% | 71.0% | XM_009935660.1 |
| PREDICTED: <i>Pan troglodytes</i> actin, alpha 1, skeletal muscle (ACTA1), mRNA | <i>Pan troglodytes</i> | 99% | 71.0% | XM_016940594.1 |
| PREDICTED: <i>Pterocles gutturalis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Pterocles gutturalis</i> | 99% | 70.9% | XM_010082746.1 |
| PREDICTED: <i>Pterocles gutturalis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Pterocles gutturalis</i> | 99% | 70.9% | XM_010082745.1 |
| PREDICTED: <i>Catharus ustulatus</i> actin alpha 2, smooth muscle (ACTA2), mRNA | <i>Catharus ustulatus</i> | 99% | 70.9% | XM_033065747.1 |
| PREDICTED: <i>Aptenodytes forsteri</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Aptenodytes forsteri</i> | 99% | 70.9% | XM_009277749.2 |
| PREDICTED: <i>Pygoscelis adeliae</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Pygoscelis adeliae</i> | 99% | 70.8% | XM_009320191.1 |
| PREDICTED: <i>Balearica regulorum gibbericeps</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Balearica regulorum gibbericeps</i> | 99% | 70.7% | XM_010305821.1 |
| PREDICTED: <i>Balearica regulorum gibbericeps</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Balearica regulorum gibbericeps</i> | 99% | 70.7% | XM_010305820.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X3, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897268.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X2, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897267.1 |
| PREDICTED: <i>Egretta garzetta</i> actin alpha 2, smooth muscle (ACTA2), transcript variant X1, mRNA | <i>Egretta garzetta</i> | 99% | 70.7% | XM_035897266.1 |
| PREDICTED: <i>Perca fluviatilis</i> actin, alpha cardiac muscle 1 (LOC120549587), mRNA | <i>Perca fluviatilis</i> | 99% | 70.7% | XM_039786604.1 |
| PREDICTED: <i>Orbicella faveolata</i> actin, cytoplasmic 1 (LOC110065202), mRNA | <i>Orbicella faveolata</i> | 99% | 70.6% | XM_020772311.1 |
| PREDICTED: <i>Fulmarus glacialis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Fulmarus glacialis</i> | 99% | 70.6% | XM_009576330.1 |
| PREDICTED: <i>Fulmarus glacialis</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Fulmarus glacialis</i> | 99% | 70.6% | XM_009576329.1 |
| PREDICTED: <i>Gymnodraco acuticeps</i> actin, alpha skeletal muscle (LOC117544084), mRNA | <i>Gymnodraco acuticeps</i> | 99% | 70.5% | XM_034213253.1 |
| PREDICTED: <i>Hipposideros armiger</i> actin, alpha 1, skeletal muscle (ACTA1), transcript variant X2, mRNA | <i>Hipposideros armiger</i> | 99% | 70.5% | XM_019626360.1 |
| PREDICTED: <i>Hipposideros armiger</i> actin, alpha 1, skeletal muscle (ACTA1), transcript variant X1, mRNA | <i>Hipposideros armiger</i> | 99% | 70.5% | XM_019626358.1 |
| PREDICTED: <i>Sturnus vulgaris</i> actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA | <i>Sturnus vulgaris</i> | 99% | 70.4% | XM_014875386.1 |
| PREDICTED: <i>Pseudochaenichthys georgianus</i> actin, alpha skeletal muscle (LOC117468060), mRNA | <i>Pseudochaenichthys georgianus</i> | 99% | 70.4% | XM_034112020.1 |
| PREDICTED: <i>Trematomus bernacchii</i> actin, alpha skeletal muscle (LOC117478450), mRNA | <i>Trematomus bernacchii</i> | 99% | 70.4% | XM_034125513.1 |
| PREDICTED: <i>Vombatus ursinus</i> actin, alpha skeletal muscle (LOC114049606), mRNA | <i>Vombatus ursinus</i> | 99% | 70.4% | XM_027870867.1 |
| PREDICTED: <i>Vombatus ursinus</i> actin, alpha skeletal muscle (LOC114049602), mRNA | <i>Vombatus ursinus</i> | 99% | 70.4% | XM_027870861.1 |
| PREDICTED: <i>Phascolarctos cinereus</i> actin, alpha 1, skeletal muscle (ACTA1), mRNA | <i>Phascolarctos cinereus</i> | 99% | 70.3% | XM_021000483.1 |
| PREDICTED: <i>Charadrius vociferus</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA | <i>Charadrius vociferus</i> | 99% | 70.3% | XM_009888704.1 |
| PREDICTED: <i>Charadrius vociferus</i> actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA | <i>Charadrius vociferus</i> | 99% | 70.3% | XM_009888702.1 |
| <u><i>Clupea harengus</i> actin alpha 1, skeletal muscle a (acta1a), mRNA</u> | <u><i>Clupea harengus</i></u> | <u>99%</u> | <u>70.3%</u> | <u>NM_001309834.1</u> |

Table S7. NCBI BLAST result for *Chromodoris annae* actin isoform amino acid sequence.

| Description | Scientific Name | Query Cover | Per. Ident | Accession |
|---|---------------------------------------|-------------|--------------|-------------------|
| actin, cytoplasmic-like [Branchiostoma floridae] | <i>Branchiostoma floridae</i> | 99% | 79.5% | XP_035697441.1 |
| unnamed protein product [Dimorphilus gyrotilatus] | <i>Dimorphilus gyrotilatus</i> | 99% | 79.1% | CAD5124374.1 |
| actin-2-like [Amblyraja radiata] | <i>Amblyraja radiata</i> | 99% | 79.1% | XP_032876180.1 |
| ACTB_G1 [Mytilus coruscus] | <i>Mytilus coruscus</i> | 99% | 79.1% | CAC5391235.1 |
| actin A3 [Haliotis iris] | Haliotis iris | 99% | 78.7% | AAX19288.1 |
| actin isoform X2 [Salvelinus namaycush] | <i>Salvelinus namaycush</i> | 99% | 78.7% | XP_038842271.1 |
| hypothetical protein HELRODRAFT_96235 [Helobdella robusta] | <i>Helobdella robusta</i> | 99% | 78.7% | XP_009030330.1 |
| actin [Crotalaria spectabilis] | <i>Crotalaria spectabilis</i> | 99% | 78.7% | CEO86982.1 |
| actin, non-muscle 6.2-like [Salvelinus alpinus] | <i>Salvelinus alpinus</i> | 99% | 78.7% | XP_023997352.1 |
| cytoplasmic actin [Hirudo medicinalis] | <i>Hirudo medicinalis</i> | 99% | 78.7% | ABC60434.1 |
| hypothetical protein HELRODRAFT_185075 [Helobdella robusta] | <i>Helobdella robusta</i> | 99% | 78.7% | XP_009025437.1 |
| cytoplasmic actin [Hirudo medicinalis] | <i>Hirudo medicinalis</i> | 99% | 78.7% | ABC60436.1 |
| actin, non-muscle 6.2 isoform X1 [Salvelinus namaycush] | <i>Salvelinus namaycush</i> | 99% | 78.7% | XP_038842264.1 |
| non-muscle actin II [Hydractinia echinata] | <i>Hydractinia echinata</i> | 99% | 78.7% | ADR10434.1 |
| actin, cytoplasmic 2 [Pseudoalteromonas sp. BMB] | <i>Pseudoalteromonas sp. BMB</i> | 99% | 78.7% | WP_069019001.1 |
| actin, cytoplasmic-like [Branchiostoma floridae] | <i>Branchiostoma floridae</i> | 99% | 78.7% | XP_035695124.1 |
| hypothetical protein FO519_010338 [Halicephalobus sp. NKZ332] | <i>Halicephalobus sp. NKZ332</i> | 99% | 78.4% | KAE9546450.1 |
| actin A2 [Haliotis iris] | Haliotis iris | 99% | 78.4% | AAX19287.1 |
| actin-2-like isoform X1 [Mizuhopecten yessoensis] | <i>Mizuhopecten yessoensis</i> | 99% | 78.4% | XP_021356756.1 |
| predicted protein [Hordeum vulgare subsp. vulgare] | <i>Hordeum vulgare subsp. vulgare</i> | 99% | 78.4% | BAJ97607.1 |
| Actin protein [Sycon ciliatum] | <i>Sycon ciliatum</i> | 99% | 78.4% | CCQ18644.1 |
| actin A1 [Haliotis iris] | Haliotis iris | 99% | 78.4% | AAX19286.1 |
| ACTB_G1 [Mytilus coruscus] | <i>Mytilus coruscus</i> | 99% | 78.4% | CAC5420182.1 |
| actin [Euagrus chioseus] | <i>Euagrus chioseus</i> | 99% | 78.4% | ABZ91664.1 |
| Actin-4 [Caenorhabditis elegans] | <i>Caenorhabditis elegans</i> | 99% | 78.4% | NP_001368079.1 |
| unnamed protein product [Spirometra erinaceieuropaei] | <i>Spirometra erinaceieuropaei</i> | 99% | 78.4% | VZI50703.1 |
| actin, non-muscle 6.2 [Thalassophryne amazonica] | <i>Thalassophryne amazonica</i> | 99% | 78.4% | XP_034027240.1 |
| PREDICTED: actin, non-muscle 6.2 [Hydra vulgaris] | <i>Hydra vulgaris</i> | 99% | 78.4% | XP_002154462.1 |
| hypothetical protein LOTGIDRAFT_193218 [Lottia gigantea] | <i>Lottia gigantea</i> | 99% | 78.4% | XP_009060604.1 |
| actin [Thecamoeba similis] | <i>Thecamoeba similis</i> | 99% | 78.4% | AAQ55801.1 |
| actin [Euagrus chioseus] | <i>Euagrus chioseus</i> | 99% | 78.4% | ABZ91662.1 |
| PREDICTED: actin, adductor muscle [Octopus bimaculoides] | <i>Octopus bimaculoides</i> | 99% | 78.4% | XP_014771927.1 |

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|---|-------------------------------------|-----|--------------|-------------------|
| Bm9237, isoform b [<i>Brugia malayi</i>] | <i>Brugia malayi</i> | 99% | 78.4% | CDP93363.1 |
| non-muscle actin 6.2 [<i>Malo kingi</i>] | <i>Malo kingi</i> | 99% | 78.4% | ACY74447.1 |
| actin, cytoplasmic 2 [<i>Escherichia coli</i>] | <i>Escherichia coli</i> | 99% | 78.4% | WP_126755788.1 |
| unnamed protein product [<i>Onchocerca ochengi</i>] | <i>Onchocerca ochengi</i> | 99% | 78.4% | VDM97777.1 |
| Actin [<i>Oesophagostomum dentatum</i>] | <i>Oesophagostomum dentatum</i> | 99% | 78.4% | KHJ95400.1 |
| hypothetical protein LOTGIDRAFT_208208 [<i>Lottia gigantea</i>] | <i>Lottia gigantea</i> | 99% | 78.4% | XP_009043796.1 |
| actin [<i>Bursaphelenchus xylophilus</i>] | <i>Bursaphelenchus xylophilus</i> | 99% | 78.4% | BAI52958.1 |
| PREDICTED: actin, non-muscle 6.2-like [<i>Hydra vulgaris</i>] | <i>Hydra vulgaris</i> | 99% | 78.4% | XP_002154696.1 |
| actin [<i>Stemonaria longa</i>] | <i>Stemonaria longa</i> | 99% | 78.0% | AFY23984.1 |
| beta-actin [<i>Cepaea nemoralis</i>] | <i>Cepaea nemoralis</i> | 99% | 78.0% | AXI69344.1 |
| beta-actin 2 [<i>Haliotis diversicolor</i>] | <i>Haliotis diversicolor</i> | 99% | 78.0% | ABY87412.1 |
| actin [<i>Haliotis diversicolor</i>] | <i>Haliotis diversicolor</i> | 99% | 78.0% | ABU86741.1 |
| actin-2 isoform X1 [<i>Pecten maximus</i>] | <i>Pecten maximus</i> | 99% | 78.0% | XP_033737032.1 |
| actin-3-like [<i>Mizuhopecten yessoensis</i>] | <i>Mizuhopecten yessoensis</i> | 99% | 78.0% | XP_021356754.1 |
| actin [<i>Mesenchytraeus solifugus</i>] | <i>Mesenchytraeus solifugus</i> | 99% | 78.0% | AOR07101.1 |
| actin [<i>Haliotis tuberculata</i>] | <i>Haliotis tuberculata</i> | 99% | 78.0% | CAJ85786.1 |
| actin [<i>Eisenia fetida</i>] | <i>Eisenia fetida</i> | 99% | 78.0% | AUS83928.1 |
| Actin actin domain containing protein [<i>Meloidogyne graminiicola</i>] | <i>Meloidogyne graminiicola</i> | 99% | 78.0% | KAF7632952.1 |
| hypothetical protein L596_016279 [<i>Steinernema carpocapsae</i>] | <i>Steinernema carpocapsae</i> | 99% | 78.0% | TKR82582.1 |
| actin-2-like [<i>Mizuhopecten yessoensis</i>] | <i>Mizuhopecten yessoensis</i> | 99% | 78.0% | XP_021356759.1 |
| ACTB_G1 [<i>Mytilus coruscus</i>] | <i>Mytilus coruscus</i> | 99% | 78.0% | CAC5419237.1 |
| hypothetical protein EGW08_007646 [<i>Elysia chlorotica</i>] | <i>Elysia chlorotica</i> | 99% | 78.0% | RUS84619.1 |
| uncharacterized protein MONBRDRAFT_37852 [<i>Monosiga brevicollis</i> MX1] | <i>Monosiga brevicollis</i> MX1 | 99% | 78.0% | XP_001747496.1 |

Gastropoda shown in bold.

Table S8. NCBI BLAST result for *Elysia viridis* actin isoform amino acid sequence.

| Description | Scientific Name | Query Cover | Per. Ident | Accession |
|---|--------------------------------|-------------|---------------|-------------------|
| actin [<i>Plakobranthus ocellatus</i>] | <i>Plakobranthus ocellatus</i> | 100% | 98.52% | GFN80144.1 |
| actin [<i>Elysia marginata</i>] | <i>Elysia marginata</i> | 100% | 98.52% | GFS25392.1 |
| RecName: Full=Actin, cytoplasmic, intermediate form; [<i>Biomphalaria pfeifferi</i>] | <i>Biomphalaria pfeifferi</i> | 100% | 97.79% | Q964E2.1 |
| actin [<i>Elysia marginata</i>] | <i>Elysia marginata</i> | 100% | 97.79% | GFR57762.1 |
| actin 1 [<i>Halisarca dujardini</i>] | <i>Halisarca dujardini</i> | 100% | 97.79% | Q5X72278.1 |
| actin [<i>Elysia marginata</i>] | <i>Elysia marginata</i> | 100% | 97.79% | GFR57757.1 |

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|--|---------------------------------|------|---------------|---------------------|
| hypothetical protein EGW08_006629 [Elysia chlorotica] | Elysia chlorotica | 100% | 97.79% | RUS85617.1 |
| act protein isoform X1 [Ciona intestinalis] | <i>Ciona intestinalis</i> | 100% | 97.42% | XP_009861333.1 |
| actin, cytoplasmic [Asterias rubens] | <i>Asterias rubens</i> | 100% | 97.42% | XP_033640031.1 |
| actin, cytoplasmic [Orbicella faveolata] | <i>Orbicella faveolata</i> | 100% | 97.42% | XP_020600429.1 |
| hypothetical protein LDENG_00240370 [Lucifuga dentata] | <i>Lucifuga dentata</i> | 100% | 97.42% | KAF7643397.1 |
| actin 4 [Halisarca dujardini] | <i>Halisarca dujardini</i> | 100% | 97.42% | QSDX72281.1 |
| beta-actin isotype 2 [Lymnaea stagnalis] | Lymnaea stagnalis | 100% | 97.42% | AOV18887.1 |
| beta actin [Doryteuthis pealeii] | <i>Doryteuthis pealeii</i> | 100% | 97.42% | AAU11523.1 |
| cytoplasmic actin [Dreissena polymorpha] | <i>Dreissena polymorpha</i> | 100% | 97.42% | AAC32224.1 |
| actin [Rapana venosa] | Rapana venosa | 100% | 97.42% | AGZ87937.1 |
| unnamed protein product [Candidula unifasciata] | Candidula unifasciata | 100% | 97.42% | CAG5123311.1 |
| actin, cytoplasmic-like [Actinia tenebrosa] | <i>Actinia tenebrosa</i> | 100% | 97.05% | XP_031571867.1 |
| Actin, cytoplasmic, intermediate form; [Biomphalaria tenagophila] | Biomphalaria tenagophila | 100% | 97.05% | Q964E0.1 |
| PREDICTED: actin, cytoplasmic [Pundamilia nyererei] | <i>Pundamilia nyererei</i> | 100% | 97.05% | XP_005754021.1 |
| cytoplasmic actin 1 [Botryllus schlosseri] | <i>Botryllus schlosseri</i> | 100% | 97.05% | CAX48981.1 |
| actin, cytoplasmic 1 [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 97.05% | XP_001637076.1 |
| actin, cytoplasmic [Exaiptasia diaphana] | <i>Exaiptasia diaphana</i> | 100% | 97.05% | XP_020916414.1 |
| hypothetical protein BaRGS_009049 [Batillaria attramentaria] | Batillaria attramentaria | 100% | 97.05% | KAG5710333.1 |
| actin, cytoplasmic [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 97.05% | XP_001621292.2 |
| actin, cytoplasmic 1-like [Sphaeramia orbicularis] | <i>Sphaeramia orbicularis</i> | 100% | 97.05% | XP_029985695.1 |
| actin, cytoplasmic [Cheilinus undulatus] | <i>Cheilinus undulatus</i> | 100% | 97.05% | XP_041642816.1 |
| PREDICTED: actin, cytoplasmic 1-like [Poecilia reticulata] | <i>Poecilia reticulata</i> | 100% | 97.05% | XP_008417682.1 |
| beta-actin [Meretrix meretrix] | <i>Meretrix meretrix</i> | 100% | 97.05% | AEK81538.1 |
| hypothetical protein BaRGS_009048 [Batillaria attramentaria] | Batillaria attramentaria | 100% | 97.05% | KAG5710332.1 |
| beta actin [Exaiptasia diaphana] | <i>Exaiptasia diaphana</i> | 100% | 97.05% | AAQ62633.1 |
| actin-like protein [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 97.05% | AGG36337.1 |
| actin, cytoplasmic [Patiria miniata] | <i>Patiria miniata</i> | 100% | 97.05% | XP_038044475.1 |
| actin, cytoplasmic isoform X1 [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 97.05% | XP_022795459.1 |
| Actin, cytoplasmic [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 97.05% | PFX22595.1 |
| actin, cytoplasmic [Sebastes umbrosus] | <i>Sebastes umbrosus</i> | 100% | 97.05% | XP_037633840.1 |
| actin, non-muscle 6.2 [Salaria fasciatus] | <i>Salaria fasciatus</i> | 100% | 97.05% | XP_029961458.1 |
| unnamed protein product [Darwinula stevensoni] | <i>Darwinula stevensoni</i> | 100% | 97.05% | CAD7248021.1 |
| beta-actin [Cepaea nemoralis] | Cepaea nemoralis | 100% | 97.05% | AXI69344.1 |
| Oidioi.mRNA.OKI2018_I69.PAR.g11455.t1.cds [Oikopleura dioica] | <i>Oikopleura dioica</i> | 100% | 97.05% | CAG5087094.1 |

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|--|--|------|---------------|-----------------------|
| unnamed protein product [Oikopleura dioica] | <i>Oikopleura dioica</i> | 100% | 97.05% | CBY24839.1 |
| actin [Plakobranthus ocellatus] | <i>Plakobranthus ocellatus</i> | 100% | 97.05% | GFN80142.1 |
| Actin, cytoplasmic, intermediate form; [Biomphalaria alexandrina] | <i>Biomphalaria alexandrina</i> | 100% | 96.68% | Q964E3.1 |
| beta-actin [Nothobranchius furzeri] | <i>Nothobranchius furzeri</i> | 100% | 96.68% | ABR86936.1 |
| actin, cytoplasmic 1 [Oreochromis niloticus] | <i>Oreochromis niloticus</i> | 100% | 96.68% | XP_003444532.1 |
| actin, cytoplasmic [Oryzias latipes] | <i>Oryzias latipes</i> | 100% | 96.68% | XP_011477986.1 |
| actin [Cerebratulus lacteus] | <i>Cerebratulus lacteus</i> | 100% | 96.68% | ANC90245.1 |
| non-muscle actin 6.2 [Malo kingi] | <i>Malo kingi</i> | 100% | 96.68% | ACY74447.1 |
| PREDICTED: actin, cytoplasmic 1-like [Nothobranchius furzeri] | <i>Nothobranchius furzeri</i> | 100% | 96.68% | XP_015815812.1 |
| Actin, cytoplasmic, intermediate form; Flags: Precursor [Planorbella trivolvis] | <i>Planorbella trivolvis</i> | 100% | 96.68% | Q964D9.1 |
| beta-actin isotype 1 [Lymnaea stagnalis] | <i>Lymnaea stagnalis</i> | 100% | 96.68% | AOV18885.1 |
| Actin, cytoplasmic, intermediate form; Flags: Precursor [Biomphalaria glabrata] | <i>Biomphalaria glabrata</i> | 100% | 96.68% | P92179.2 |
| hypothetical protein LOTGIDRAFT_205506 [Lottia gigantea] | <i>Lottia gigantea</i> | 100% | 96.68% | XP_009065998.1 |
| hypothetical protein CCH79_00016215 [Gambusia affinis] | <i>Gambusia affinis</i> | 100% | 96.68% | PWA24173.1 |
| hypothetical protein OJAV_G00095600 [Oryzias javanicus] | <i>Oryzias javanicus</i> | 100% | 96.68% | RVE68811.1 |

Gastropoda shown in bold.

Table S9. NCBI BLAST result for *Aplysia californica* actin nucleotide sequence.

| Description | Scientific Name | Query Cover | Per. Ident |
|---|-------------------------------------|-------------|---------------|
| Aplysia californica actin (LOC100533345), mRNA | <i>Aplysia californica</i> | 100% | 100.0% |
| PREDICTED: Aplysia californica actin, cytoplasmic (LOC106013368), mRNA | <i>Aplysia californica</i> | 100% | 96.4% |
| Littorina littorea actin (Act1) mRNA, complete cds | <i>Littorina littorea</i> | 100% | 88.9% |
| PREDICTED: Megalops cyprinoides actin, beta 2 (actb2), mRNA | <i>Megalops cyprinoides</i> | 100% | 88.3% |
| PREDICTED: Acipenser ruthenus actin, beta 1 (actb1), mRNA | <i>Acipenser ruthenus</i> | 100% | 88.3% |
| Rapana venosa actin (Act1) mRNA, complete cds | <i>Rapana venosa</i> | 100% | 88.3% |
| Acipenser dabryanus beta actin mRNA, partial cds | <i>Acipenser dabryanus</i> | 100% | 88.0% |
| Haliotis midae clone Hdd.c148 microsatellite sequence | <i>Haliotis midae</i> | 100% | 88.0% |
| Haliotis diversicolor clone HDr4CJ470 beta-actin 2 mRNA, complete cds | <i>Haliotis diversicolor</i> | 100% | 88.0% |
| PREDICTED: Acipenser ruthenus actin, cytoplasmic 2 (LOC117418240), mRNA | <i>Acipenser ruthenus</i> | 100% | 87.9% |
| PREDICTED: Pangasianodon hypophthalmus actin, beta 2 (actb2), mRNA | <i>Pangasianodon hypophthalmus</i> | 100% | 87.9% |
| Danio rerio actin, beta 2 (actb2), mRNA | <i>Danio rerio</i> | 100% | 87.9% |
| Danio rerio bactin2, mRNA (cDNA clone MGC:172104 IMAGE:7912932), complete cds | <i>Danio rerio</i> | 100% | 87.9% |

PREDICTED: *Esox lucius* actin, beta 2 (actb2), mRNA
PREDICTED: *Cyprinus carpio* actin, cytoplasmic 1 (LOC109051881), mRNA
Spinibarbus denticulatus beta-actin mRNA, complete cds
Danio rerio bactin2, mRNA (cDNA clone MGC:85665 IMAGE:6960309), complete cds
Danio rerio beta actin mRNA, complete cds
Haliotis diversicolor actin mRNA, complete cds
PREDICTED: ***Biomphalaria glabrata*** actin, adductor muscle (LOC106058166), mRNA
Haliotis tuberculata mRNA for actin (actin gene) from haemocyte cells
Urechis unicinctus beta-actin mRNA, complete cds
PREDICTED: *Esox lucius* actin, beta 1 (actb1), mRNA
Acipenser dabryanus beta-actin mRNA, complete cds
Carassius auratus B-actin mRNA for beta-actin, complete cds
Danio rerio bactin2, mRNA (cDNA clone MGC:192911 IMAGE:100061397), complete cds
Danio rerio bactin2, mRNA (cDNA clone MGC:56040 IMAGE:3820122), complete cds
Danio rerio beta-actin mRNA, complete cds
Morulus calbasu beta-actin mRNA, complete cds
Danio rerio actin, beta 1 (actb1), mRNA
PREDICTED: *Cyprinus carpio* actin, cytoplasmic 1 (LOC109073280), transcript variant X2, mRNA
PREDICTED: *Cyprinus carpio* actin, cytoplasmic 1 (LOC109073280), transcript variant X1, mRNA
Sinocyclocheilus anshuiensis actin, cytoplasmic 1 (LOC107692666), mRNA
Sinocyclocheilus anshuiensis actin, cytoplasmic 1-like (LOC107702535), transcript variant X2, mRNA
Sinocyclocheilus anshuiensis actin, cytoplasmic 1-like (LOC107702535), transcript variant X1, mRNA
Danio rerio bactin1, mRNA (cDNA clone MGC:77623 IMAGE:6996683), complete cds
Aplysia californica actin, muscle (LOC100533357), mRNA
Placopecten magellanicus actin mRNA, complete cds
PREDICTED: *Anguilla anguilla* actin, cytoplasmic 2 (LOC118216518), mRNA
PREDICTED: *Sinocyclocheilus anshuiensis* actin, cytoplasmic 1 (LOC107703420), mRNA
Crassostrea gigas genome assembly, linkage group: LG7
PREDICTED: *Carassius auratus* actin, cytoplasmic 1 (LOC113044540), mRNA
PREDICTED: *Sinocyclocheilus rhinoceros* actin, cytoplasmic 1 (LOC107725373), mRNA
PREDICTED: *Sinocyclocheilus rhinoceros* actin, cytoplasmic 1 (LOC107722944), mRNA
PREDICTED: *Cyprinodon variegatus* actin, beta (actb), mRNA
Rhodeus uyekii beta-actin mRNA, complete cds
Onychostoma macrolepis beta-actin mRNA, complete cds

| | | |
|-------------------------------------|------|--------------|
| <i>Esox lucius</i> | 100% | 87.8% |
| <i>Cyprinus carpio</i> | 100% | 87.8% |
| <i>Spinibarbus denticulatus</i> | 100% | 87.8% |
| <i>Danio rerio</i> | 100% | 87.8% |
| <i>Danio rerio</i> | 100% | 87.8% |
| <i>Haliotis diversicolor</i> | 100% | 87.8% |
| <i>Biomphalaria glabrata</i> | 99% | 87.7% |
| <i>Haliotis tuberculata</i> | 100% | 87.6% |
| <i>Urechis unicinctus</i> | 100% | 87.6% |
| <i>Esox lucius</i> | 100% | 87.5% |
| <i>Acipenser dabryanus</i> | 100% | 87.5% |
| <i>Carassius auratus</i> | 100% | 87.5% |
| <i>Danio rerio</i> | 100% | 87.5% |
| <i>Danio rerio</i> | 100% | 87.5% |
| <i>Danio rerio</i> | 100% | 87.5% |
| <i>Labeo calbasu</i> | 100% | 87.4% |
| <i>Danio rerio</i> | 100% | 87.4% |
| <i>Cyprinus carpio</i> | 100% | 87.4% |
| <i>Cyprinus carpio</i> | 100% | 87.4% |
| <i>Sinocyclocheilus anshuiensis</i> | 100% | 87.4% |
| <i>Sinocyclocheilus anshuiensis</i> | 100% | 87.4% |
| <i>Sinocyclocheilus anshuiensis</i> | 100% | 87.4% |
| <i>Danio rerio</i> | 100% | 87.4% |
| <i>Aplysia californica</i> | 100% | 87.4% |
| <i>Placopecten magellanicus</i> | 100% | 87.3% |
| <i>Anguilla anguilla</i> | 100% | 87.2% |
| <i>Sinocyclocheilus anshuiensis</i> | 100% | 87.2% |
| <i>Crassostrea gigas</i> | 99% | 87.2% |
| <i>Carassius auratus</i> | 100% | 87.1% |
| <i>Sinocyclocheilus rhinoceros</i> | 100% | 87.1% |
| <i>Sinocyclocheilus rhinoceros</i> | 100% | 87.1% |
| <i>Cyprinodon variegatus</i> | 100% | 87.1% |
| <i>Rhodeus uyekii</i> | 100% | 87.1% |
| <i>Onychostoma macrolepis</i> | 100% | 87.1% |

| | | | |
|--|------------------------------------|------|--------------|
| PREDICTED: Colossoma macropomum actin, beta 1 (actb1), mRNA | <i>Colossoma macropomum</i> | 100% | 87.1% |
| Danio rerio bactin1, mRNA (cDNA clone MGC:192419 IMAGE:100060865), complete cds | <i>Danio rerio</i> | 100% | 87.1% |
| Danio rerio bactin1, mRNA (cDNA clone MGC:55989 IMAGE:3819668), complete cds | <i>Danio rerio</i> | 100% | 87.1% |
| PREDICTED: Pangasianodon hypophthalmus actin, beta 1 (actb1), transcript variant X2, mRNA | <i>Pangasianodon hypophthalmus</i> | 100% | 87.1% |
| PREDICTED: Pangasianodon hypophthalmus actin, beta 1 (actb1), transcript variant X1, mRNA | <i>Pangasianodon hypophthalmus</i> | 100% | 87.1% |
| PREDICTED: Pomacea canaliculata actin, adductor muscle (LOC112575079), mRNA | <i>Pomacea canaliculata</i> | 99% | 87.1% |
| PREDICTED: Merops nubicus actin, cytoplasmic 1 (LOC103775609), transcript variant X5, mRNA | <i>Merops nubicus</i> | 99% | 87.1% |
| PREDICTED: Merops nubicus actin, cytoplasmic 1 (LOC103775609), transcript variant X1, mRNA | <i>Merops nubicus</i> | 99% | 87.1% |

Gastropoda shown in bold.

Table S10. NCBI BLAST results for *Aplysia californica* actin amino acid sequence.

| Description | Scientific Name | Query Cover | Per. Ident |
|---|---------------------------------|-------------|---------------|
| actin [Aplysia californica] | <i>Aplysia californica</i> | 100% | 100.0% |
| actin, cytoplasmic [Aplysia californica] | <i>Aplysia californica</i> | 100% | 98.1% |
| actin [Littorina littorea] | <i>Littorina littorea</i> | 100% | 97.8% |
| actin, cytoplasmic [Pomacea canaliculata] | <i>Pomacea canaliculata</i> | 100% | 97.8% |
| actin, cytoplasmic [Octopus sinensis] | <i>Octopus sinensis</i> | 100% | 97.8% |
| Actin, cytoplasmic, intermediate form [Biomphalaria tenagophila] | <i>Biomphalaria tenagophila</i> | 100% | 97.4% |
| Actin, cytoplasmic, intermediate form [Biomphalaria obstructa] | <i>Biomphalaria obstructa</i> | 100% | 97.4% |
| actin [Rapana venosa] | <i>Rapana venosa</i> | 100% | 97.4% |
| Actin, cytoplasmic, intermediate form [Biomphalaria pfeifferi] | <i>Biomphalaria pfeifferi</i> | 100% | 97.4% |
| beta-actin isotype 2 [Lymnaea stagnalis] | <i>Lymnaea stagnalis</i> | 100% | 97.4% |
| hypothetical protein LOTGIDRAFT_227913 [Lottia gigantea] | <i>Lottia gigantea</i> | 100% | 97.4% |
| actin, cytoplasmic isoform X1 [Lingula anatina] | <i>Lingula anatina</i> | 100% | 97.4% |
| Actin, cytoplasmic, intermediate form [Biomphalaria alexandrina] | <i>Biomphalaria alexandrina</i> | 100% | 97.0% |
| actin, cytoplasmic-like [Actinia tenebrosa] | <i>Actinia tenebrosa</i> | 100% | 97.0% |
| Actin, cytoplasmic, intermediate form [Planorbella trivolvis] | <i>Planorbella trivolvis</i> | 100% | 97.0% |
| actin, cytoplasmic [Orbicella faveolata] | <i>Orbicella faveolata</i> | 100% | 97.0% |
| cytoplasmic actin [Dreissena polymorpha] | <i>Dreissena polymorpha</i> | 100% | 97.0% |
| actin [Diplodon chilensis] | <i>Diplodon chilensis</i> | 100% | 97.0% |
| PREDICTED: actin, cytoplasmic [Octopus bimaculoides] | <i>Octopus bimaculoides</i> | 100% | 97.0% |
| cytoplasmic actin [Pinctada fucata] | <i>Pinctada fucata</i> | 100% | 97.0% |
| beta-actin [Cepaea nemoralis] | <i>Cepaea nemoralis</i> | 100% | 97.0% |
| actin, cytoplasmic 1 [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 96.7% |

| | | | |
|--|-------------------------------------|------|--------------|
| actin, cytoplasmic [Asterias rubens] | <i>Asterias rubens</i> | 100% | 96.7% |
| act protein isoform X1 [Ciona intestinalis] | <i>Ciona intestinalis</i> | 100% | 96.7% |
| hypothetical protein LOTGIDRAFT_205506 [Lottia gigantea] | <i>Lottia gigantea</i> | 100% | 96.7% |
| actin, cytoplasmic [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 96.7% |
| actin, cytoplasmic 1 [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 96.7% |
| beta-actin isotype 1 [Lymnaea stagnalis] | <i>Lymnaea stagnalis</i> | 100% | 96.7% |
| Actin, cytoplasmic, intermediate form [Biomphalaria glabrata] | <i>Biomphalaria glabrata</i> | 100% | 96.7% |
| beta-actin [Sinanodonta woodiana] | <i>Sinanodonta woodiana</i> | 100% | 96.7% |
| beta-actin [Meretrix meretrix] | <i>Meretrix meretrix</i> | 100% | 96.7% |
| actin-2 [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 96.7% |
| actin beta/gamma 1 [Paragonimus westermani] | <i>Paragonimus westermani</i> | 100% | 96.7% |
| Actin, cytoplasmic [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 96.7% |
| actin-like protein [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 96.7% |
| actin, cytoplasmic isoform X1 [Stylophora pistillata] | <i>Stylophora pistillata</i> | 100% | 96.7% |
| beta actin [Doryteuthis pealeii] | <i>Doryteuthis pealeii</i> | 100% | 96.7% |
| Actin-2 isoform 1 [Schistosoma japonicum] | <i>Schistosoma japonicum</i> | 100% | 96.7% |
| PREDICTED: actin, cytoplasmic [Pundamilia nyererei] | <i>Pundamilia nyererei</i> | 100% | 96.3% |
| actin, cytoplasmic 2-like [Petromyzon marinus] | <i>Petromyzon marinus</i> | 100% | 96.3% |
| actin, cytoplasmic 1-like [Sphaeramia orbicularis] | <i>Sphaeramia orbicularis</i> | 100% | 96.3% |
| hypothetical protein LDENG_00240370 [Lucifuga dentata] | <i>Lucifuga dentata</i> | 100% | 96.3% |
| hypothetical protein LOTGIDRAFT_202971 [Lottia gigantea] | <i>Lottia gigantea</i> | 100% | 96.3% |
| predicted protein [Nematostella vectensis] | <i>Nematostella vectensis</i> | 100% | 96.3% |
| PREDICTED: actin, cytoplasmic [Acropora digitifera] | <i>Acropora digitifera</i> | 100% | 96.3% |
| PREDICTED: actin, cytoplasmic 1-like [Poecilia formosa] | <i>Poecilia formosa</i> | 100% | 96.3% |
| actin, cytoplasmic-like [Monopterus albus] | <i>Monopterus albus</i> | 100% | 96.3% |
| actin [Galaxea fascicularis] | <i>Galaxea fascicularis</i> | 100% | 96.3% |
| actin, cytoplasmic 1 [Oreochromis niloticus] | <i>Oreochromis niloticus</i> | 100% | 95.9% |
| actin, cytoplasmic [Oryzias latipes] | <i>Oryzias latipes</i> | 100% | 95.9% |
| PREDICTED: actin, cytoplasmic 1-like [Nothobranchius furzeri] | <i>Nothobranchius furzeri</i> | 100% | 95.9% |
| beta-actin [Nothobranchius furzeri] | <i>Nothobranchius furzeri</i> | 100% | 95.9% |
| actin, cytoplasmic 2 [Oryzias melastigma] | <i>Oryzias melastigma</i> | 100% | 95.9% |
| actin [Cerebratulus lacteus] | <i>Cerebratulus lacteus</i> | 100% | 95.9% |
| PREDICTED: actin, cytoplasmic 1-like [Poecilia reticulata] | <i>Poecilia reticulata</i> | 100% | 95.9% |

Gastropoda shown in bold.

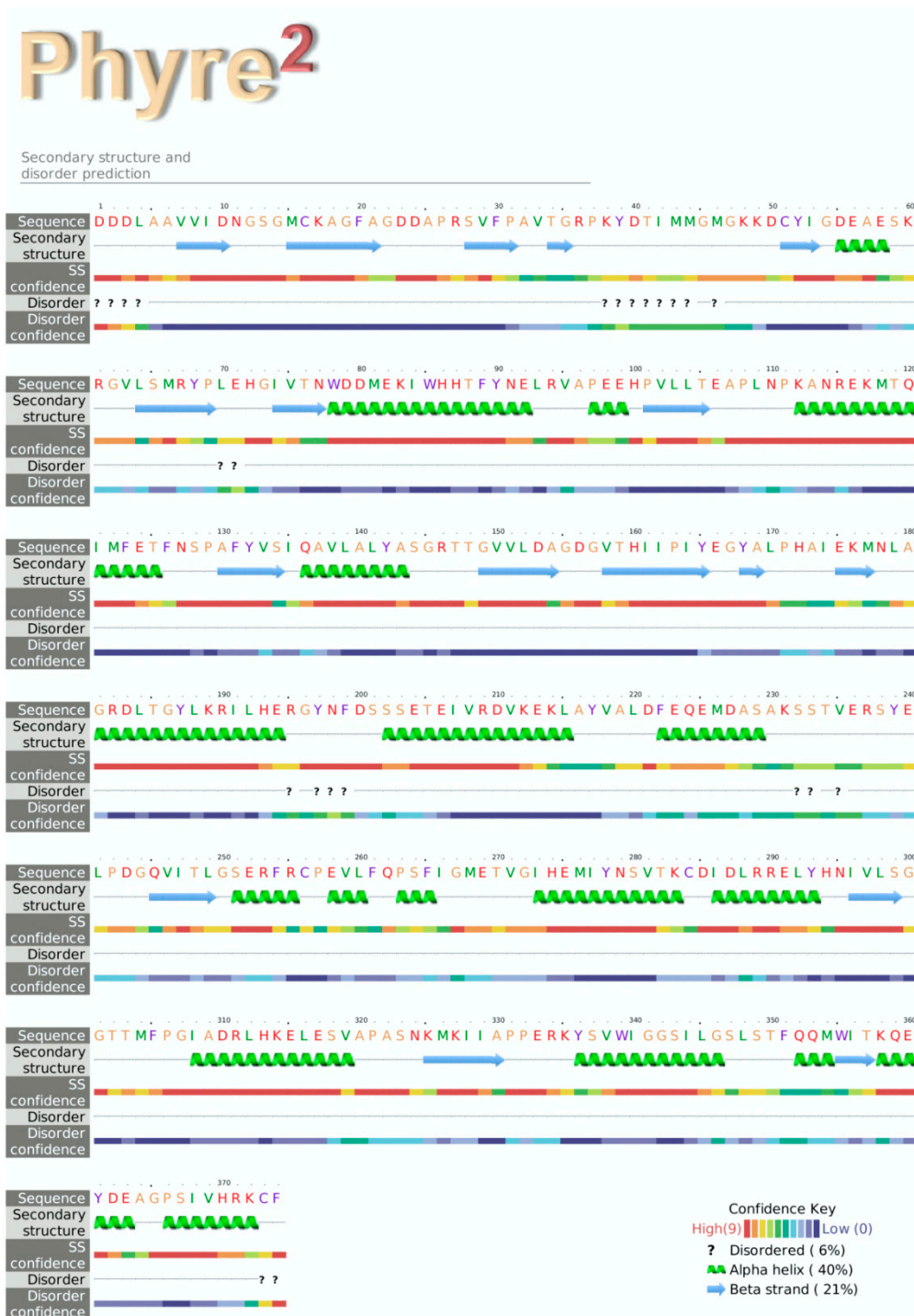


Figure S9. Phyre2 prediction of secondary structure and disorder of the in silico *Chromodoris/Armina*-actin-hybrid-model.