

## Supplementary Materials for

### Formation of the Isthmus of Panama

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Published 17 August 2016, *Sci. Adv.* **2**, e1600883 (2016)

DOI: 10.1126/sciadv.1600883

#### The PDF file includes:

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- table S3. Median and 95% HPD intervals of the time at which members of clades spanning the Isthmus of Panama were separated from each other as estimated by BEAST (151) from phylogenies calibrated by fossils at one or more nodes.
- table S4. Kimura two-parameter distance between mitochondrial genes of sister clades on either side of the Isthmus of Panama.
- text S1. Estimation of date of splitting from molecular divergence.
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#### Other Supplementary Material for this manuscript includes the following:

(available at [advances.sciencemag.org/cgi/content/full/2/8/e1600883/DC1](http://advances.sciencemag.org/cgi/content/full/2/8/e1600883/DC1))

- video S1 (.mov format). Large rafts form on the Río Chagres, Gamboa, Republic of Panama, on the 8th of December, 2010.



NORTH AMERICA



CARIBBEAN  
SEA

*Isthmus of Panama*

PACIFIC  
OCEAN

SOUTH  
AMÉRICA

**fig. S1. The Isthmus of Panama in Tropical America.**

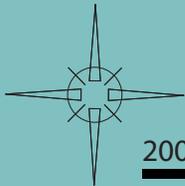
C A R I B B E A N S E A

Geologically-young  
El Valle volcano

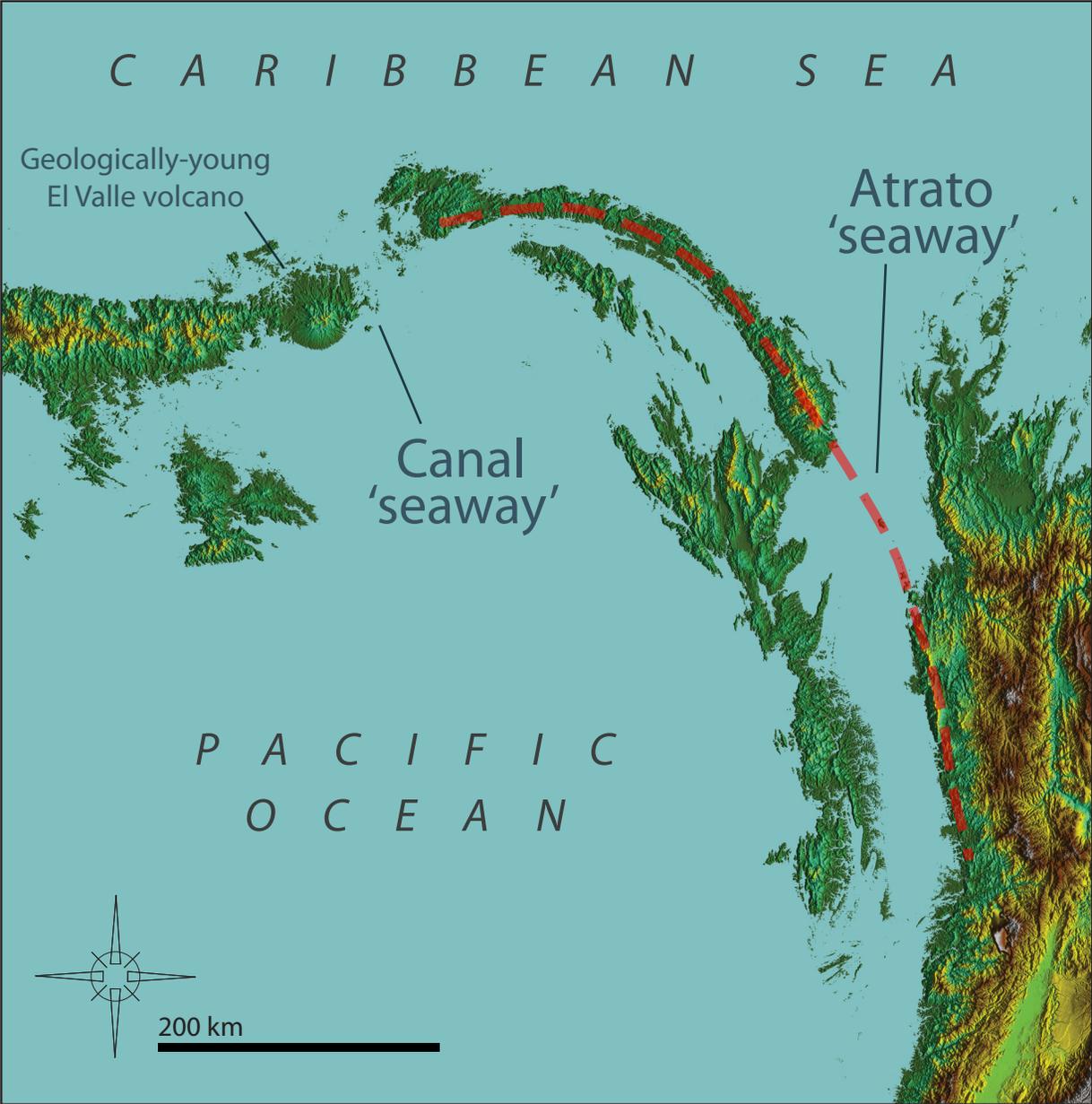
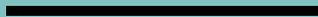
Atrato  
'seaway'

Canal  
'seaway'

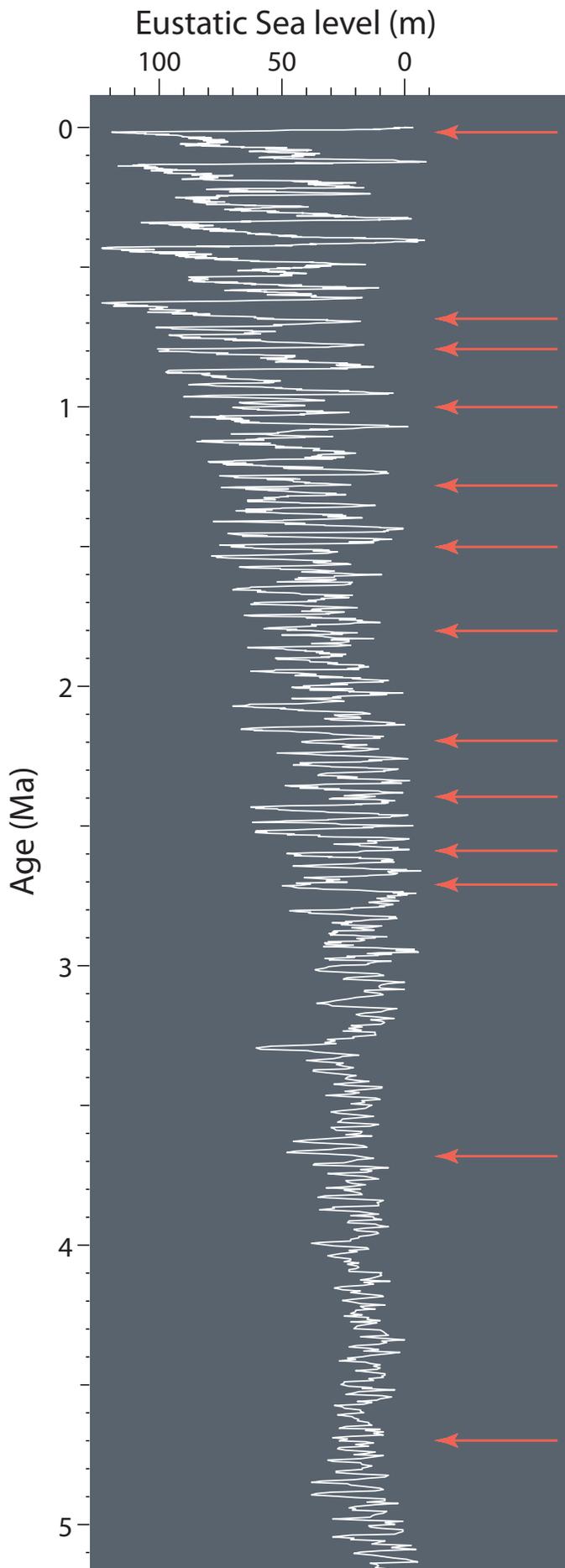
P A C I F I C  
O C E A N



200 km



**fig. S2. The current day Isthmus of Panama submerged under 150 m of relative sea-level rise.** Interoceanic connections form through hypothetical Canal and Atrato seaways. Such relative sea level rise is easily attained by reversing the moderate rates of tectonic uplift over the last few million years (Fig. 1). Dashed line follows the Darién-Acandí-Cuchillo-Debeiba structure. This is not a paleogeographic reconstruction as it does not compensate for tectonic movements, pre-emergence deposition, post-emergence erosion or volcanic activity. For example, the El Valle volcano is a geologically-young structure and its absence would have permitted greater interoceanic connection through the Canal 'seaway' (cf. 15)



0.125 Ma *Nasua*, *Lontra*, *Procyon*, *Pteronura*, *Ozotoceros*, *Eulamaops*, Leporidae (*Sylvilagus*), *Equus*, *Leopardus*, *Homo* to S.A.  
Recent: Soricidae (*Cryptotis*), Geomyidae (*Orythogeomys*), Heteromyidae (*Heteromys*) and Sciuridae (*Microsciurus*) may have entered S.A. at this time or subsequently

0.7 Ma *Morenelephus*, *Paraceros*, *Tayassu*, *Puma* to S.A.

0.8 Ma *Didelphis* to N.A.

1.0 Ma Ursidae (*Arctotherium*), Felidae (*Panthera*), Machairodontidae (*Smilodon*), Canidae (*Canis*, *Procyon*), Tayassuidae (*Catagonus*), Cervidae (*Antifer*, *Epieuryceros*), Camelidae (*Lama*), Tapiridae (*Tapirus*),

1.3 Ma *Nothrotheriops* to N.A. Gomphotheriidae (*Cuvieronius*, *Notiomastodon*), Mustelidae (*Conepatus*, *Galictis*), Cricetidae (*Bolomys*, *Eligmodontia*, *Graomys*, *Lundomys*, *Necromys*, *Oxymycterus*, *Phyllotis*, *Holochilius*, *Calomys*) (1Ma) to S.A.

1.5 Ma *Mixotoxodon* to N.A.

1.8 Ma *Myrmecophaga* to N.A.

2.2 Ma *Hydrochoerus* to N.A. *Equus* (~2-1 Ma) to S.A.

2.4 Ma *Holmesina*, *Dasybus*, *Pampatherium*, *Pachyarmatherium*, *Eremotherium* to N.A. Equidae (*Hippidium*), ?Gomphotheriidae, Mustelidae (*Stipanicia*) to S.A.

2.6 Ma *Erethizon* to N.A. Mustelidae, Canidae to S.A.

2.7 Ma *Glyptotherium* to N.A.

~4-3.3 Ma *Nechoerus* to N.A.

Tayassuidae (*Platygonus*) and Camelidae (*Hemiauchenia*) to S.A.

3.7 Ma. *Pampatherium* to N.A.

4.7 Ma *Glossotherium*, *Plaina* to N.A. *Megalonyx* to S.A

~5Ma *Titanis* to N.A. Sigmodontine rodents to S.A.

**fig. S3. Timing of successful terrestrial dispersals between the American continents as observed in the occurrence of fossil remains in the rock record of North and South America. Sea level data from (131).**

**video S1. Large rafts form on the Río Chagres, Gamboa, Republic of Panama, on the 8th of December, 2010. Footage from Collin McMillan.**

**table S1. Estimates of rates of uplift for the Panama Arc relative to sea level, using changes in estimated median depths and median ages of sedimentary units from studies (28, 135-149). See below for methodology.**

| Region         | Unit 1   | Unit 2  | Median age Unit 1 (Ma) | Median age Unit 2 (Ma) | Median depth Unit 1 (m) | Median depth Unit 2 (m) | Median uplift rate relative to sea level (m Myr <sup>-1</sup> ) |
|----------------|--|---|------------------------|------------------------|-------------------------|-------------------------|---|
| Bocas del Toro | Punta Alegre Fm., Valiente Peninsula   | Valiente Fm., Llorona Hill, Valiente Peninsula                                  | 19.9                   | 15.4                   | 1500                    | 800                     | 155.6   |
| Bocas del Toro | Valiente Fm., Toro Point, PPP site 3136  | Valiente Fm., Toro Point, PPP sites 2730/31                                     | 15.4                   | 12.6                   | 400                     | 400                     | 0.0   |
| Bocas del Toro | Isla Popa, Deer Island   | Isla Popa, Punta Laurel   | 15                     | 13.6                   | 800                     | 400                     | 285.7   |
| Bocas del Toro | Nancy Point Fm., Tobabe Point, Valiente Peninsula                                    | Shark Hole Pt Fm, Bruno Bluff, Valiente Peninsula                               | 6.1                    | 3.7                    | 400                     | 175                     | 93.8  |
| Bocas del Toro | Cayo Agua Fm., NE Cayo Agua island   | Cayo Agua Fm, NW Cayo Agua island   | 4.8                    | 3.7                    | 60                      | 30                      | 27.3  |
| Bocas del Toro | Escudo de Veraguas Fm, NE part of Escudo de Veraguas island, PPP sites CJ88-30-11/12 | Escudo de Veraguas Fm, NW part of Escudo de Veraguas island                     | 3.7                    | 1.9                    | 75                      | 75                      | 0.0   |
| Bocas del Toro | Escudo de Veraguas Fm, NW part of Escudo de Veraguas island                          | Modern  | 1.9                    | 0                      | 125                     | 0                       | 65.8  |
| Bocas del Toro | Swan Cay island, off Isla Colon island, base of bluffs                               | Modern  | 1.3                    | 0                      | 100                     | 1                       | 76.2  |
| Bocas del Toro | Bastimentos island, Fish Hole, Western Sequence                                      | Modern  | 2.7                    | 0                      | 88                      | 2                       | 31.9  |
| Limon          | Uscari Fm, early Miocene *   | Uscari Fm, middle Miocene #   | 17.7                   | 15.8                   | 800                     | 400                     | 210.5   |
| Limon          | Uscari Fm, e. middle Miocene, middle part of section of Cassell & Sen Gupta 1989     | Uscari Fm, m. middle Miocene, upper part of section of Cassell & Sen Gupta 1989 | 15.8                   | 13.9                   | 400                     | 150                     | 131.6   |
| Limon          | Uscari Fm, m. middle Miocene, upper part of section of Cassell & Sen Gupta, 1989     | Uscari Fm, Rio Sandbox and Carbon Dos sections                                  | 13.9                   | 6.7                    | 150                     | 400                     | -34.7   |
| Limon          | Rio Banano Fm, Rio Banano, Quitaria section  | Rio Banano Fm, Rio Banano, type section near Bomba                              | 3.7                    | 2.8                    | 30                      | 20                      | 11.1  |
| Limon          | Rio Banano Fm, Rio Banano, type section near Bomba                                   | Modern  | 2.8                    | 0                      | 20                      | 5                       | 5.4   |
| Limon          | Moin Fm, type section in unnamed creek in Cangrejos community                        | Modern  | 1.8                    | 0                      | 200                     | 10                      | 105.6   |
| Limon          | Moin Fm, E reef, Lomas del Mar Mbr   | Modern  | 1.8                    | 0                      | 75                      | -50                     | 69.4  |
| Panama Canal   | Gatun Fm, lower part   | Gatun Fm, middle-upper part   | 11.6                   | 9                      | 25                      | 25                      | 0.0   |
| Panama         | Gatun Fm, upper part   | Chagres Fm, Toro Point Mbr  | 9                      | 7                      | 25                      | 350                     | -162.5  |

|              |   |  |      |      |      |      |        |  |
|--------------|---|--|------|------|------|------|--------|--|
| Canal        |   |  |      |      |      |      |        |  |
| Panama Canal | Gatun Fm, upper part                          | Chagres Fm, Rio Indio facies             | 9    | 7    | 25   | 65   | -20.0  |  |
| Panama Canal | Chagres Fm, Rio Indio facies                  | Chagres Fm, main facies                  | 7    | 6.1  | 65   | 350  | -316.7 |  |
| Darien       | Membrillo River, Clarita Fm                   | Membrillo River, Tapaliza Fm             | 15.6 | 12   | 1750 | 1000 | 208.3  |  |
| Darien       | Membrillo River, Tapaliza Fm                  | Membrillo River, Membrillo Fm            | 12   | 10.3 | 1000 | 1000 | 0.0    |  |
| Darien       | Membrillo River, Membrillo Fm                 | Membrillo River, Chucunaque Fm           | 10.3 | 6.4  | 1000 | 325  | 173.1  |  |
| Darien       | Tuquesa River, Clarita Fm                     | Tuquesa River, Tapaliza Fm               | 15   | 12.7 | 1000 | 1000 | 0.0    |  |
| Darien       | Tuquesa River, Tapaliza Fm                    | Tuquesa River, Tuira Fm, lower part      | 12.7 | 10.3 | 1000 | 45   | 397.9  |  |
| Darien       | Tuquesa River, Tuira Fm, upper part           | Tuquesa River, Yaviza Fm                 | 10.3 | 9    | 15   | 15   | 0.0    |  |
| Darien       | Tuquesa River, Yaviza Fm                      | Tuquesa River, Chucunaque Fm, lower part | 9    | 7.1  | 15   | 15   | 0.0    |  |
| Darien       | Tuquesa River, Chucunaque Fm, base of section | Tuquesa River, Chucunaque Fm, upper part | 7.1  | 6.4  | 15   | 150  | -192.9 |  |
| Darien       | Tupisa River, Tuira Fm                        | Tupisa River, Yaviza Fm                  | 9.6  | 9    | 15   | 15   | 0.0    |  |
| Darien       | Tupisa River, Yaviza Fm                       | Tupisa River, Chucunaque Fm              | 9    | 7.2  | 15   | 105  | -50.0  |  |
| Darien       | Yaviza section, Tapaliza Fm, lower part       | Yaviza section, Tapaliza Fm, upper part  | 12.8 | 11   | 1000 | 325  | 375.0  |  |
| Darien       | Yaviza section, Tapaliza Fm, upper part       | Yaviza section, Tuira Fm, lower part     | 11   | 9.9  | 325  | 105  | 200.0  |  |
| Darien       | Yaviza section, Tuira Fm, upper part          | Yaviza section, Yaviza Fm                | 9.9  | 9    | 15   | 325  | -344.4 |  |
| Darien       | Tuira River, Tuira Fm                         | Tuira River, Yaviza Fm                   | 9.9  | 9    | 325  | 105  | 244.4  |  |
| Burica       | Burica Fm                                     | Armuelles Fm                             | 2.6  | 0.4  | 1300 | 10   | 586.4  |  |

### *Methodology and notes*

Rates of uplift relative to sea level were calculated using changes in the estimated depths of deposition of 38 sedimentary units in five sedimentary basins (Limon in Costa Rica, Bocas del Toro, Panama Canal basin, Darien and Burica in Panama).

Ages of deposition of sedimentary units were estimated using planktic foraminifera and calcareous nannoplankton while depth at deposition were estimated from assemblages of benthic foraminifera, both using references below. Rates of uplift relative to sea level for each sequence (defined as an older sedimentary unit to a younger sedimentary sequence) was calculated as:

(median depth of unit 1)-(medium depth of unit 2) / (median age of unit 1)-(median age of unit 2)

where unit 1 is the older of the two sedimentary units.

When younger sedimentary units were not available we used the modern (i.e. age = 0 Ma, depth = 0 m). This approach is appropriate because all fossil stratigraphic units lay at or close to modern day sea level.

Mean rates of uplift relative to sea level were then calculated for each million year bin by including all the rates of uplift relative to sea level from sequences which crossed that time bin. Likewise, standard deviation was calculated for each mean in each time bin.

\* = Lowest part of section of Cassel and Sen Gupta (135). # = Middle part of section from Cassel and Sen Gupta (135). § = Upper part of section from Cassel and Sen Gupta (135).

**table S2. Compilation of Late Paleocene–Late Eocene ages for the Colombian Andes plotted in Fig. 2.** Entries are organized in order of increased ages. Only data from the interval of interest (~59 to 33 Ma) relative to Montes et al.'s (2015) claims are reported. Radiometric ages compiled from various sources including references 1-3 below (see notes and references). Vast majority of radiometric ages in this critical interval fall outside of the Panama-Chocó Block litho-structural domain (see Fig. 2).

| Lithologic Unit   | Age (Ma) | Error $\pm$ Myr | Method    | Lat.  | Lon.   | Ref.  |
|---|----------|-----------------|-----------|-------|--------|-------|
| Dacite  | 33.9     | 0.7             | K/Ar wr   | 2.56  | -76.69 | (132) |
| Mandé Batholith (granodiorite)                              | 34       |                 | K/Ar Bt   | 5.72  | -76.35 | (132) |
| Grupo Diabásico (dolerite)                                  | 34       |                 | K/Ar      | 3.27  | -76.62 | (132) |
| Santa Marta Batholith. (granodiorite)                       | 34.2     | 1.6             | Ar/Ar Kfs | 11.24 | -74.02 | (132) |
| Dibulla Gneiss (anorthosite)                                | 35       | 3               | Ar/Ar Hb  | 10.74 | -74.08 | (132) |
| Cocha Río Téllez Migmatitic Complex (gneissic granodiorite) | 35       | 0.4             | Ar/Ar Hb  | 0.81  | -77.33 | (132) |
| Santa Marta Schist (amphibolic schist)                      | 36.2     | 5.1             | K/Ar Hb   | 11.28 | -74.15 | (132) |
| Paja Fm. (mineralized vein)                                 | 36.4     | 0.1             | Ar/Ar Ms  | 5.64  | -74.14 | (132) |
| Cocha Río Téllez Migmatitic Complex (gneissic granodiorite) | 36.4     | 0.6             | Ar/Ar Hb  | 0.81  | -77.33 | (132) |
| Santa Cecilia La Equis Complex (porphyritic basalt)         | 36.7     | 11.5            | Ar/Ar     | 6.74  | -76.39 | (132) |
| Patía 29-Ra-002   | 37.1     | 1.7             | Ar/Ar     | 1.98  | -77.15 | (132) |
| Paja Fm. (mineralized vein)                                 | 37.3     | 0.1             | Ar/Ar wr  | 5.64  | -74.14 | (132) |
| Socorro Stock (granodiorite)                                | 37.8     | 1.7             | K/Ar Bt   | 10.79 | -74.03 | (132) |
| Santa Marta Batholith (granodiorite)                        | 38.65    | 0.55            | Ar/Ar.Kfs | 11.24 | -74.02 | (132) |
| Santa Marta Schist. Fm. Concha (phyllite)                   | 38.7     | 3.4             | K/Ar wr   | 11.31 | -74.13 | (132) |
| Acandí Batholith (quartz-diorite)                           | 38.9     | 3               | K/Ar Ser  | 8.53  | -77.42 | (132) |
| Timbiquí Fm. (andesite)                                     | 38.9     | 4.3             | K/Ar      | 2.29  | -77.65 | (132) |
| Río Napi Intrusives (Hb diorite)                            | 39       | 2               | K/Ar      | 2.49  | -77.48 | (132) |
| Grupo Diabásico (dolerite)                                  | 39       |                 | K/Ar      | 3.27  | -76.62 | (132) |
| Grupo Diabásico   | 39.65    | 3.47            | Ar/Ar Hb  | 1.33  | -77.46 | (132) |
| Grupo Diabásico (lava)                                      | 40       | 2               | K/Ar wr   | 12.23 | -71.69 | (132) |
| Piedrancha Batholith (granodiorite)                         | 40.5     | 3               | K/Ar Bt   | 1.23  | -77.73 | (150) |
| Cocha Río Téllez Migmatitic Complex (gneissic granodiorite) | 40       | 0.5             | Ar/Ar Hb  | 0.81  | -77.33 | (132) |
| Grupo Diabásico (dolerite)                                  | 40       |                 | K/Ar      | 3.27  | -76.62 | (132) |
| Santa Marta Batholith (granodiorite)                        | 40.16    | 1.4             | Ar/ArKfs  | 11.28 | -73.90 | (132) |
| Santa Marta Batholith (granodiorite)                        | 40.2     | 1.45            | Ar/ArKfs  | 11.28 | -73.90 | (132) |
| Santa Marta Batholith (granodiorite)                        | 40.39    | 0.27            | Ar/ArKfs  | 11.28 | -73.90 | (132) |
| Santa Schist Marta. Cinto Fm. (phyllite)                    | 40.9     | 4.7             | K/Ar wr   | 11.25 | -74.18 | (132) |
| Nudillales Stock (quartz-monzonite)                         | 41       | 3               | K/Ar wr   | 7.04  | -76.32 | (132) |
| Timbiquí Fm. (andesite)                                     | 41       | 1               | K/Ar      | 2.20  | -77.68 | (132) |
| Los Cholos-Napi River Pluton (Hb bearing quartz-diorite)    | 41       | 4               | K/Ar      | 2.46  | -77.50 | (132) |
| Basalt  | 41.4     | 8.6             | Ar/Ar PI  | 6.02  | -76.26 | (132) |
| Llanitos Latiandesite                                       | 41.5     | 1.8             | K/Ar wr   | 7.07  | -76.41 | (132) |
| Timbiquí Fm. (andesite)                                     | 41.7     | 1.2             | K/Ar      | 2.40  | -77.57 | (132) |
| Santa Marta Batholith (granodiorite)                        | 41.78    | 0.83            | Ar/Ar Kfs | 11.27 | -74.09 | (132) |
| Patía 29-Ra-002   | 41.9     | 0.7             | Ar/Ar     | 1.98  | -77.15 | (132) |
| Amaime Fm.  | 42       | 13              | Ar/Ar wr  | 3.70  | -76.18 | (132) |
| Balsitas Pluton (andesite dike)                             | 42.6     | 1.3             | K/Ar      | 2.17  | -77.70 | (132) |
| Santa Marta Schist (biotite schist)                         | 42.6     | 1.7             | K/Ar Bt   | 10.99 | -74.14 | (132) |
| Mandé Batholith (porphyritic dacite)                        | 42.7     | 0.9             | K/Ar Ser  | 6.70  | -76.50 | (132) |
| Pórfido Pantanos (porphyritic dacite)                       | 42.7     | 0.9             | K/Ar Bt   | 6.42  | -76.30 | (150) |
| Río Napi Intrusives (Hb-bearing gabbro)                     | 43       | 0.4             | K/Ar      | 2.53  | -77.45 | (132) |
| Basalt  | 43.1     | 0.4             | Ar/Ar PI  | 6.02  | -76.26 | (132) |

|   |       |      |                        |       |        |       |
|---|-------|------|------------------------|-------|--------|-------|
| Santa Marta Batholith (granodiorite)                | 43.57 | 0.48 | Ar/Ar Kfs              | 11.27 | -74.09 | (132) |
| Buriticá Andesite (andesite, porphyritic diorite)   | 43.8  | 4.3  | K/Ar wr                | 6.70  | -75.91 | (132) |
| Santa Marta Batholith (granodiorite)                | 43.91 | 0.45 | Ar/Ar Bt               | 11.28 | -73.90 | (132) |
| Santa Marta Batholith (granodiorite)                | 44    | 0.77 | Ar/Ar Bt               | 11.28 | -73.90 | (132) |
| Río Napi Intrusives (Hb-bearing tonalite)           | 44    | 4    | K/Ar                   | 2.49  | -77.49 | (132) |
| Timbiquí Fm. (andesite)                             | 44    | 1    | K/Ar                   | 2.18  | -77.70 | (132) |
| Santa Marta Schist (amphibolic schist)              | 44.1  | 2.7  | K/Ar Hb                | 11.22 | -73.89 | (132) |
| Santa Marta Batholith (quartz-diorite)              | 44.1  | 1.6  | K/Ar Bt                | 11.29 | -73.97 | (132) |
| Mandé Batholith (tonalite)                          | 44.6  | 0.9  | U/Pb LA–MC– ICP–MS Zrn | 6.73  | -76.52 | (132) |
| Los Azules (ophiolite sequence + pillow lavas)      | 44.7  | 6    | K/Ar wr                | 1.9   | -77.00 | (150) |
| Mandé Batholith (tonalite)                          | 44.8  | 1.0  | Ar/Ar Hb               | 6.81  | -76.59 | (132) |
| Sevilla Complex (schist)                            | 44.8  | 0.4  | Ar/Ar Bt               | 11.26 | -73.62 | (132) |
| Mandé Batholith. (tonalite)                         | 45.3  | 1.2  | U/Pb LA–MC– ICP–MS Zrn | 6.72  | -76.52 | (132) |
| Grupo Diabásico (lava)                              | 46    | 3    | K/Ar wr                | 3.51  | -76.53 | (150) |
| Santa Marta Batholith (granodiorite)                | 46.01 | 0.41 | Ar/Ar Bt               | 11.24 | -74.02 | (132) |
| Dibulla Gneiss (anorthosite)                        | 46.1  | 1.4  | Ar/Ar Hb               | 10.74 | -74.08 | (132) |
| Santa Marta Batholith (granodiorite)                | 46.32 | 0.67 | Ar/Ar Bt               | 11.24 | -74.02 | (132) |
| Timbiquí Fm. (dike, andesite)                       | 46.7  | 2    | K/Ar                   | 2.18  | -77.70 | (132) |
| Sabaletas Stock (gabbro, diorite)                   | 46.9  | 8.1  | Ar/Ar Hb               | 3.82  | -76.60 | (132) |
| Grupo Diabásico (dolerite)                          | 47    |      | K/Ar                   | 3.27  | -76.62 | (132) |
| Mandé Batholith (tonalite)                          | 47.1  | 2.5  | K/Ar Hb                |       |        | (150) |
| Santa Marta Schist (amphibolic schist)              | 47.4  | 2.4  | K/Ar Hb                | 11.12 | -74.05 | (132) |
| Santa Marta Batholith (granodiorite)                | 47.75 | 0.60 | Ar/Ar Hb               | 11.28 | -73.90 | (132) |
| Esquistos de Santa Marta (pegmatite)                | 47.8  | 1.9  | K/Ar Ms                | 11.26 | -74.15 | (132) |
| Parashi Stock (quartzdiorite)                       | 48    | 4    | K/Ar Hb                | 12.23 | -71.74 | (132) |
| Balsitas Pluton (tonalite)                          | 48    | 1    | K/Ar                   | 2.17  | -77.69 | (132) |
| Santa Marta Batholith (granodiorite)                | 48.04 | 0.77 | Ar/Ar Hb               | 11.24 | -74.02 | (132) |
| Acandí Batholith (tonalite)                         | 48.1  | 1    | K/Ar Hb                | 8.20  | -77.24 | (150) |
| Acandí Batholith (tonalite)                         | 48.1  | 1    | K/Ar Ser               | 8.46  | -77.36 | (132) |
| Acandí Batholith (tonalite)                         | 48.1  | 2    | K/Ar Ser               | 8 20  | -77.24 | (150) |
| Santa Marta Batholith (granodiorite)                | 48.33 | 0.78 | Ar/Ar Hb               | 11.24 | -74.02 | (132) |
| Santa Marta Batholith (granodiorite)                | 48.34 | 0.90 | Ar/Ar Hb               | 11.28 | -73.90 | (132) |
| Timbiquí Fm. (porphyritic andesite)                 | 48.4  | 4.8  | K/Ar                   | 2.29  | -77.64 | (132) |
| Buriticá Pluton (quartzdiorite)                     | 48.4  | 1.8  | K/Ar Bt                | 11.17 | -73.73 | (132) |
| Santa Marta Batholith (quartzdiorite)               | 48.8  | 1.7  | K/Ar Hb                | 11.29 | -73.97 | (132) |
| Grupo Diabásico (pillow lava)                       | 49.4  | 9.8  | K (R)                  | 1.6   | -77.4  | (150) |
| El Bosque Batholith (granodiorite)                  | 49.1  | 1.7  | K/Ar Bt                | 4.44  | -75.08 | (150) |
| Santa Marta Batholith (granodiorite)                | 49.45 | 0.75 | Ar/Ar Bt               | 11.27 | -74.09 | (132) |
| Gneis de Dibulla (anorthosite)                      | 49.8  | 1.1  | Ar/Ar Bt               | 10.74 | -74.08 | (132) |
| Gabro de Rodrigo (Hb-Px bearing gabbro)             | 49.9  | 0.2  | Ar/Ar Pl               | 6.12  | -72.34 | (132) |
| Santa Marta Batholith (granodiorite-tonalite)       | 50.12 | 0.76 | U/Pb LA–ICP–MS Zrn     | 11.28 | -73.90 | (132) |
| Santa Marta Batholith (granodiorite)                | 50.42 | 1.05 | Ar/Ar Hb               | 11.27 | -74.09 | (132) |
| Santa Marta Batholith (granodiorite-tonalite)       | 50.6  | 1.7  | U/Pb LA–ICP–MS Zrn     | 11.31 | -73.94 | (132) |
| Santa Marta Batholith (granodiorite)                | 50.66 | 0.87 | Ar/Ar Hb               | 11.27 | -74.09 | (132) |
| Santa Cecilia La Equis Complex (porphyritic basalt) | 50.7  | 2.0  | Ar/Ar volcanic glass   | 6.74  | -76.39 | (132) |
| Timbiquí Fm. (andesite)                             | 50.7  | 2    | K/Ar                   | 2.18  | -77.70 | (132) |
| Plutón de Buriticá (tonalite- quartz-diorite)       | 50.8  | 1.5  | U/Pb LA–ICP–MS Zrn     | 11.18 | -73.73 | (132) |
| Santa Marta Batholith (granodiorite)                | 50.91 | 0.8  | Ar/Ar Bt               | 11.27 | -74.09 | (132) |
| Plutón El Salto (pegmatite)                         | 51    | 1    | K/Ar                   | 2.21  | -77.66 | (132) |
| Esquistos de Santa Marta (amphibolic schist)        | 51    | 3.6  | K/Ar Hb                | 11.01 | -74.12 | (132) |
| Timbiquí Fm. (porphyritic andesite)                 | 51.5  | 1.5  | K/Ar                   | 2.21  | -77.69 | (132) |
| Arquí Complex (garnet-bearing amphibolite)          | 51.6  | 3.3  | Ar/Ar Hb               | 4.38  | -75.72 | (132) |
| Gabbronorite  | 51.7  | 3.9  | Ar/Ar wr               | 6.58  | -76.59 | (132) |
| Santa Marta Batholith, (applitite dike)             | 52.28 | 0.65 | U/Pb LA–ICP–MS Zrn     | 11.14 | -74.12 | (132) |
| Gabbronorite  | 52.7  | 3.2  | Ar/Ar wr               | 6.58  | -76.59 | (132) |
| El Hatillo Stock (quartzdiorite)                    | 53    | 1.8  | K/Ar Bt                | 5.19  | -75.00 | (150) |
| Río Napi Intrusives (Hb-bearing tonalite)           | 53    | 5    | K/Ar                   | 2.52  | -77.43 | (132) |
| Grupo Diabásico (pillow lava)                       | 53.2  | 4.6  | K/Ar wr                | 1.6   | -77.4  | (150) |

|  |       |      |                        |       |        |       |
|--|-------|------|------------------------|-------|--------|-------|
| Santa Marta Batholith (aplite dike)                  | 53.3  | 1.0  | U/Pb LA-ICP-MS Zrn     | 11.24 | -74.06 | (132) |
| Timbiquí Fm. (andesite)                              | 53.4  | 3    | K/Ar                   | 2.19  | -77.71 | (132) |
| Gabbronorite   | 53.6  | 2.9  | Ar/Ar wr               | 6.58  | -76.59 | (132) |
| Gneis de Dibulla (anorthosite)                       | 53.8  | 0.7  | Ar/Ar Bt               | 10.74 | -74.08 | (132) |
| Sevilla Complex                                      | 53.9  | 0.5  | Ar/Ar Bt               | 11.26 | -73.62 | (132) |
| Plutón Tucuriquita (granodiorite)                    | 54    | 2.2  | K/Ar Bt                | 10.68 | -74.08 | (132) |
| Sevilla Complex. (schist)                            | 54.1  | 0.7  | Ar/Ar Bt               | 11.26 | -73.62 | (132) |
| Gneis de Dibulla (anorthosite)                       | 54.3  | 1.9  | Ar/Ar Hb               | 10.74 | -74.08 | (132) |
| Esquistos de Santa Marta. Rodadero Fm. (amphibolite) | 54.3  | 2.7  | K/Ar Hb                | 11.20 | -74.21 | (132) |
| Esquistos de Jambaló (glaucophane blue schist)       | 54.5  | 1.6  | Ar/Ar Pg               | 2.77  | -76.33 | (132) |
| Gneis de Dibulla (anorthosite)                       | 54.5  | 0.8  | Ar/Ar Bt               | 10.74 | -74.08 | (132) |
| El Hatillo Stock (quartz-diorite)                    | 54.6  | 0.7  | U/Pb LA-MC- ICP-MS Zrn | 5.17  | -74.97 | (30)  |
| Santa Marta Batholith (aplite dike)                  | 54.69 | 0.69 | U/Pb LA-ICP-MS Zrn     | 11.27 | -74.09 | (132) |
| Gneis de Dibulla (anorthosite)                       | 54.7  | 4    | Ar/Ar Hb               | 10.74 | -74.08 | (132) |
| Mandé Batholith (tonalite)                           | 54.7  | 1.3  | K/Ar Hb                | 7.05  | -76.75 | (132) |
| Pórfido de Murindó (porphyry tonalite)               | 54.7  | 1.3  | K/Ar Bt                | 7.03  | -76.45 | (150) |
| Florencia Stock (quartz-diorite)                     | 54.9  | 1.9  | K/Ar Bt                | 5.53  | -75.05 | (132) |
| Florencia Stock (quartz-diorite)                     | 54.9  | 1.9  | K/Ar Bt                | 5.37  | -75.01 | (150) |
| Santa Bárbara Batholith (diorite)                    | 55    | 1    | K/Ar Bt                | 3.37  | -76.13 | (132) |
| Santa Cecilia La Equis Complex (porphyritic basalt)  | 55.1  | 1.5  | Ar/Ar                  | 6.74  | -76.39 | (132) |
| Santa Marta Batholith (granodiorite- (tonalite)      | 55.05 | 1.05 | U/Pb LA-ICP-MS Zrn     | 11.20 | -74.10 | (132) |
| Santa Marta Batholith (granodiorite-tonalite)        | 55.34 | 0.60 | U/Pb LA-ICP-MS Zrn     | 11.17 | -74.17 | (132) |
| Gneis de Dibulla (anorthosite)                       | 55.4  | 0.7  | Ar/Ar Bt               | 10.74 | -74.08 | (132) |
| Santa Marta Batholith (granodiorite-tonalite)        | 55.52 | 0.34 | U/Pb LA-ICP-MS Zrn     | 11.27 | -74.09 | (132) |
| Sonsón Batholith (leucogranite)                      | 55.8  | 1    | U/Pb LA-MC-ICP-MS Zrn  | 5.66  | -75.20 | (132) |
| Dike (andesite-dacite)                               | 55.9  | 2    | K/Ar Ser               | 6.45  | -74.63 | (132) |
| Santa Marta Batholith (dike)                         | 55.9  | 0.29 | U/Pb LA-ICP-MS Zrn     | 11.21 | -74.24 | (132) |
| Piedrancha Batholith (micro diorite)                 | 57.7  | 3    | K/Ar Bt                | 1.12  | -77.86 | (150) |
| Pórfido Rio Manso (quartz diorite. porphyry)         | 58    | 10   | K/Ar Hb                | 4.11  | -75.25 | (150) |
| Stock Mainzales                                      | 59.8  | 0.7  | U/Pb LA-MC-ICP-MS Zrn  | 5.12  | -75.29 | (30)  |

## text S1. Estimation of date of splitting from molecular divergence

We used data from fossil calibrated molecular phylogenies published until 2015. Median and 95% HPDs of node ages were taken from the original publications if BEAST (151, 152) was used in divergence dating analyses. To avoid circularity we compiled molecular divergence dates from datasets that did not rely on the final closure of the Isthmus of Panama for calibration. Because we are interested in the time of the most recent common ancestor (TMRCA) of species, rather than of particular genes, we further restricted our choice to phylogenies that were based on a combination of DNA regions.

Our final dataset was compiled from 17 studies, and contains information on divergence times for 37 splits (table S3), 12 from fishes, 13 from gastropods, 10 from bivalves, and 2 from echinoids. In two cases, a new BEAST (v. 1.8.2) analysis was performed, because previous analyses did not use BEAST, or additional sequences were available. PartitionFinder v1.1.1 (153) was used to determine best partitioning scheme and model sequence evolution for each partition. The BEAST analysis used an uncorrelated lognormal relaxed clock (154). Details of each re-analysis were as follows.

Genus *Nerita*: The original analysis (155) employed (156) to infer divergence dates. The partitioned dataset was analyzed using exponential priors on fossil calibrations. We used Frey and Vermeij (155) fossil calibrations as minimum offsets for the exponential distributions, and specified a mean of the exponential distribution that produced a 95% HPD which included the next oldest node on the phylogeny, as estimated in the original paper. Dates were estimated based on Frey and Vermeij (155) tree topology with branch lengths estimated by BEAST. The analysis was run three times, each with 50,000,000 generations, sampling every 1,000<sup>th</sup> tree. Convergence of the posterior distribution was assessed using Tracer v.1.5 (157), with ESS values >250 indicating successful convergence.

Arcid bivalves: We combined and re-analyzed the data of Marko (158) and Marko and Moran (159). A new nucleotide alignment was produced that incorporated data from COI and H3. Exponential priors were placed on the fossil calibrations with distributions encompassing those described in Marko (158). We re-estimated the topology as well as the divergence times. The analysis was run three times for 50,000,000 generations, sampling every 1,000 trees. Convergence of the posterior distribution was assessed using Tracer v.1.5 with ESS values >250 indicating successful convergence. Convergence of the tree topology was assessed using the “cumulative” and “compare” functions implemented in AWTY (160).

**table S3. Median and 95% HPD intervals of the time at which members of clades spanning the Isthmus of Panama were separated from each other as estimated by BEAST (151) from phylogenies calibrated by fossils at one or more nodes.** Clades; 1 = Teleostei, 2 = Gastropoda, 3 = Bivalvia, 4 = Echinodermata. Contractions of gene names: CYTB: Cytochrome Oxidase B; 16S: Large subunit rDNA; 12S: Small subunit rDNA; COI: Cytochrome Oxidase I; ND3: NADH dehydrogenase 3; S7II: ribosomal protein involved in S16 assembly; ETS2: transcription factor involved in cell proliferation; TMO-4C4: Anonymous protein-coding locus; MYH6: cardiac muscle myosin heavy chain 6; RAG1 and RAG2: recombination activating gene 1 and 2; Rh: Rhodopsin; BMP4: bone morphogenetic protein 4; PTR: Pulmonary tumor resistance gene; ENC1: Ectodermal-neural context 1; SREB: Brain super conserved receptor; GLYT: Glycosyltransferase; ATPSa: ATP Synthetase subunit alpha; H3: Histone 3.

| Pair   | Clade | Number on Fig. 3 | Median estimate (Ma) | Low 95% HPD (Ma) | High 95% HPD (Ma) | Genes                               | Ref.  |
|--|-------|------------------|----------------------|------------------|-------------------|-------------------------------------|-------|
| <i>Mellita quinquiesperforata</i> vs. ( <i>M. notabilis</i> , <i>M. kanakoffi</i> )  | 4     | 1                | 3.21                 | 2.51             | 3.91              | COI 16S 28S                         | (161) |
| <i>Chaetodon humeralis</i> vs. <i>C. ocellatus</i>   | 1     | 2                | 3.40                 | 1.80             | 5.40              | CYTB S7II ETS2                      | (162) |
| <i>Mycteroperca jordani</i> vs. ( <i>M. bonaci</i> , <i>M. venenosa</i> )  | 1     | 3                | 3.58                 | 1.90             | 5.51              | COI 16S TMO-4C4 S7                  | (163) |
| <i>Conus gladiator</i> vs. <i>C. mus</i>   | 2     | 4                | 4.10                 | 2.90             | 5.80              | 16S calmodulin                      | (164) |
| <i>Conus ermineus</i> vs. <i>C. purpurascens</i>   | 2     | 5                | 4.20                 | 1.50             | 7.50              | 16S calmodulin                      | (164) |
| <i>Scarus hoefleri</i> vs. <i>S. perrico</i>   | 1     | 6                | 4.82                 | 2.46             | 7.70              | 16S S7                              | (163) |
| <i>Balistes caprisacus</i> vs. <i>B. polylepis</i>   | 1     | 7                | 4.97                 | 3.14             | 7.13              | COI CYTB MYH6 RAG1 Rh               | (165) |
| ( <i>Mellita longifissa</i> , <i>M. grantii</i> ) vs. ( <i>M. isometra</i> , <i>M. tenuis</i> , <i>M. quinquiesperforata</i> ) | 4     | 8                | 5.46                 | 4.40             | 6.50              | COI 16S 28S                         | (161) |
| <i>Chromis alta</i> vs. <i>C. enchrysur</i>  | 1     | 9                | 5.57                 | 3.03             | 8.81              | 12S 16S ND3 COI CYTB RAG1 RAG2 BMP4 | (166) |
| <i>Dallocardia senticosum</i> vs. <i>Acrosterigma magnum</i>   | 3     | 10               | 6.00                 | 2.00             | 13.00             | 16S H3 28S                          | (167) |
| <i>Stegastes rectifraenum</i> vs. <i>S. imbricatus</i>   | 1     | 11               | 6.19                 | 3.17             | 9.63              | 12S 16S ND3 RAG1 RAG2 BMP4          | (166) |
| <i>Sargocentron suborbitalis</i> vs. <i>S. vexillarium</i>   | 1     | 12               | 6.20                 | 3.90             | 10.80             | COI MYH6 PTR ENC1 RAG1 SREB GLYT    | (168) |
| <i>Microspathodon chrysurus</i> vs. <i>M. dorsalis</i>   | 1     | 13               | 6.56                 | 2.75             | 11.53             | 12S 16S ND3 RAG1 RAG2 BMP4          | (166) |
| <i>Calamus brachysomus</i> vs. <i>C. nodosus</i>   | 1     | 14               | 8.00                 | 2.00             | 16.00             | COI CYTB 16S RAG1 Rh                | (169) |

|   |   |    |       |       |       |  |                       |
|---|---|----|-------|-------|-------|--|-----------------------|
| <i>Plectrypops lima</i> vs. <i>P. retrospinis</i>   | 1 | 15 | 8.10  | 4.80  | 11.60 | COI MYH6 PTR<br>ENC1 RAG1<br>SREB GLYT | (168)                 |
| <i>Littoraria irrorata</i> vs. <i>L. variegata</i>  | 2 | 16 | 8.50  | 4.50  | 12.50 | COI 12S 28S                            | (170)                 |
| <i>Pomacanthus zonipectus</i> vs. ( <i>P. paru</i> , <i>P. arcuatus</i> )   | 1 | 17 | 8.60  | 5.00  | 12.3  | 12S 16S S7                             | (171)                 |
| <i>Arcopsis adamsi</i> vs. <i>A. solida</i>   | 3 | 18 | 8.87  | 3.78  | 15.36 | COI H3                                 | (152,<br>158,<br>159) |
| <i>Conus perplexus</i> vs. <i>C. puncticulatus</i>  | 2 | 19 | 8.90  | 5.00  | 19.00 | 16S calmodulin                         | (164)                 |
| <i>Conus regius</i> vs. ( <i>C. bartschii</i> , <i>C. brunneus</i> )  | 2 | 20 | 9.20  | 5.00  | 14.90 | 16S calmodulin                         | (164)                 |
| <i>Barbatia candida</i> vs. <i>B. reeveana</i>  | 3 | 21 | 9.54  | 3.39  | 16.62 | COI H3                                 | (152,<br>158,<br>159) |
| <i>Stegastes flavilatus</i> vs. ((( <i>S. diencaeus</i> , <i>S. fuscus</i> ), <i>S. adustus</i> ), ( <i>S. variabilis</i> , <i>S. leucostictus</i> )) | 1 | 22 | 9.61  | 6.56  | 13.18 | 12S 16S ND3<br>COI RAG1 RAG2<br>BMP4   | (166)                 |
| ( <i>Echinolittorina apicina</i> , <i>E. paytensis</i> ) vs. <i>E. risei</i>  | 2 | 23 | 10.36 | 6.83  | 13.96 | COI 12S 28S                            | (164)                 |
| ( <i>Echinolittorina aspera</i> , <i>E. dubiosa</i> , <i>E. tenuistriata</i> ) vs. <i>E. interrupta</i>   | 2 | 24 | 10.75 | 7.39  | 14.35 | COI 12S 28S                            | (164)                 |
| ( <i>Echinolittorina modesta</i> , <i>E. conspersa</i> ) vs. ( <i>E. ziczac</i> , <i>E. ziczac</i> (= <i>E. soroziczac</i> ))                         | 2 | 25 | 10.87 | 7.52  | 14.42 | COI 12S 28S                            | (164)                 |
| <i>Barbatia illota</i> vs. <i>B. tenera</i>   | 3 | 26 | 11.23 | 5.09  | 18.87 | COI H3                                 | (152,<br>158,<br>159) |
| <i>Trachycardium egmontianum</i> vs. <i>Phlogocardium belcheri</i>  | 3 | 27 | 12.00 | 5.50  | 20.00 | 16S H3 28S                             | (167)                 |
| <i>Laevicardium pictum</i> vs. <i>L. elenense</i>   | 3 | 28 | 12.50 | 5.00  | 22.50 | 16S H3 28S                             | (167)                 |
| <i>Papyridea aspersa</i> vs. <i>P. semisulcata</i>  | 3 | 29 | 16.00 | 8.00  | 24.50 | 16S H3 28S                             | (167)                 |
| ( <i>Bulla gouldiana</i> , <i>B. punctulata</i> ) vs. <i>B. mabillei</i>  | 2 | 30 | 16.80 | 7.20  | 31.70 | COI 16S 28S                            | (172)                 |
| <i>Nerita scabricosta</i> vs. ( <i>N. peloronta</i> , <i>N. versicolor</i> )  | 2 | 31 | 19.29 | 16.00 | 24.24 | COI 16S ATPSa                          | (155)                 |
| <i>Anadara chemnitzii</i> vs. <i>A. nux</i>   | 3 | 32 | 20.95 | 10.69 | 32.21 | COI H3                                 | (152,<br>158,<br>159) |
| <i>Amercardia media</i> vs. ( <i>Apiocardia obovalis</i> , <i>Trigoniocardia granifera</i> )  | 3 | 33 | 21.00 | 9.00  | 30.00 | 16S H3 28S                             | (167)                 |
| <i>Echinolittorina galapagensis</i> vs. ( <i>E. tuberculata</i> , <i>E. vermeiji</i> , <i>E. miliaris</i> , <i>E. helenae</i> , <i>E. grandiosa</i> ) | 2 | 34 | 21.31 | 16.39 | 26.09 | COI 12S 28S                            | (164)                 |

|   |   |    |       |       |       |               |                       |
|---|---|----|-------|-------|-------|---------------|-----------------------|
| <i>Nerita funiculata</i> , vs. ( <i>N. tessellata</i> ,<br><i>N. fulgurans</i> , <i>N. senegalensis</i> )       | 2 | 35 | 21.80 | 17.13 | 28.16 | COI 16S ATPSa | (155)                 |
| <i>Littoraria rosewateri</i> vs. <i>L. tessellata</i>   | 2 | 36 | 24.20 | 15.00 | 32.50 | COI 12S 28S   | (170)                 |
| <i>Littoraria nebulosa</i> vs. ( <i>L. varia</i> , <i>L. zebra</i> , <i>L. irrorata</i> , <i>L. variegata</i> ) | 2 | 37 | 25.70 | 18.00 | 33.00 | COI 12S 28S   | (170)                 |
| <i>Arca mutabilis</i> vs. <i>A. imbricata</i>   | 3 | 38 | 27.50 | 12.38 | 44.42 | COI H3        | (152,<br>158,<br>159) |

**table S4. Kimura two-parameter distance between mitochondrial genes of sister clades on either side of the Isthmus of Panama.** Comparisons based on the control region were excluded, as this region is known to evolve at much faster rates. When sister clades included subclades, mean distance is shown. Age of separation of pairs in which phylogenies were calibrated by fossils are derived from BEAST (table S3). Age of separation of pairs in which there is no fossil calibration are estimated from average taxon-specific rates derived from other pairs with fossil-calibrated phylogenies. Age of separation of crustaceans, in which there are no fossil-calibrated phylogenies, are estimated from average rates over all taxa with fossil calibrated phylogenies

| Taxon / clade   | Mean divergence of all genes | Divergence rate from fossil calibration | Age of separation | COI   | ATP8 | ATP6 | CytB  | COII | COIII | ND1 | ND2 | ND3   | ND4 | ND4L | ND5 | ND6 | 12S  | 16S  | ref.  |            |
|---|------------------------------|---|-------------------|-------|------|------|-------|------|-------|-----|-----|-------|-----|------|-----|-----|------|------|-------|------------|
|   | %                            | %/Myr                                   | Ma                | %     | %    | %    | %     | %    | %     | %   | %   | %     | %   | %    | %   | %   | %    | %    |       |            |
| <b>Calibration from fossils</b>   |                              |   |                   |       |      |      |       |      |       |     |     |       |     |      |     |     |      |      |       |            |
| <b>Fishes</b>   |                              |   |                   |       |      |      |       |      |       |     |     |       |     |      |     |     |      |      |       |            |
| <i>Chaetodon humeralis</i> vs. <i>Chaetodon ocellatus</i>   | 10.25                        | 3.01                                    | 3.40              |       |      |      | 10.25 |      |       |     |     |       |     |      |     |     |      |      | (162) |            |
| <i>Mycteroperca jordani</i> vs. ( <i>M. bonaci</i> , <i>M. venenosa</i> )   | 2.72                         | 0.76                                    | 3.58              | 4.84  |      |      |       |      |       |     |     |       |     |      |     |     | 1.61 | 1.70 | (163) |            |
| <i>Scarus hoefleri</i> vs. <i>Scarus perrico</i>  | 3.16                         | 0.66                                    | 4.82              |       |      |      |       |      |       |     |     |       |     |      |     |     |      | 3.16 | (163) |            |
| <i>Balistes caprisicus</i> vs. <i>Balistes polylepis</i>  | 10.87                        | 2.19                                    | 4.97              | 11.11 |      |      | 10.63 |      |       |     |     |       |     |      |     |     |      |      | (165) |            |
| <i>Chromis alta</i> vs. <i>Chromis enchrysur</i>  | 3.60                         | 0.65                                    | 5.57              | 5.81  |      |      | 7.91  |      |       |     |     | 2.26  |     |      |     |     |      | 1.22 | 0.80  | (166)      |
| <i>Stegastes rectifraenum</i> vs. <i>Stegastes imbricatus</i>   | 4.30                         | 0.70                                    | 6.19              |       |      |      |       |      |       |     |     | 5.14  |     |      |     |     |      | 4.08 | 3.69  | (166)      |
| <i>Sargocentron suborbitalis</i> vs. <i>Sargocentron vexillarium</i>  | 5.61                         | 0.90                                    | 6.20              | 5.61  |      |      |       |      |       |     |     |       |     |      |     |     |      |      |       | (168)      |
| <i>Microspathodon chrysurus</i> vs. <i>Microspathodon dorsalis</i>  | 4.31                         | 0.66                                    | 6.56              |       |      |      |       |      |       |     |     | 8.22  |     |      |     |     |      | 2.03 | 2.68  | (166)      |
| <i>Calamus brachysomus</i> vs. <i>Calamus nodosus</i>   | 9.97                         | 1.25                                    | 8.00              | 9.69  |      |      | 17.20 |      |       |     |     |       |     |      |     |     |      |      | 3.02  | (169)      |
| <i>Plectrypops lima</i> vs. <i>Plectrypops retrospinis</i>  | 9.26                         | 1.14                                    | 8.10              | 9.26  |      |      |       |      |       |     |     |       |     |      |     |     |      |      |       | (168)      |
| <i>Pomacanthus zonipectus</i> vs. ( <i>Pomacanthus paru</i> , <i>Pomacanthus arcuatus</i> )   | 2.10                         | 0.24                                    | 8.60              |       |      |      |       |      |       |     |     |       |     |      |     |     |      | 2.10 |       | (171)      |
| <i>Stegastes flavilatus</i> vs. ((( <i>S. diencaeus</i> , <i>S. fuscus</i> ), <i>S. adustus</i> ), ( <i>S. variabilis</i> , <i>S. leucostictus</i> )) | 10.53                        | 1.10                                    | 9.61              | 9.97  |      |      |       |      |       |     |     | 18.95 |     |      |     |     |      | 6.55 | 6.66  | (166)      |
| Median  | 4.96                         | 0.83                                    | 6.20              | 9.26  | -    | -    | 10.44 | -    | -     | -   | -   | 6.68  | -   | -    | -   | -   | -    | 2.07 | 3.02  |            |
| Minimum   | 2.10                         | 0.24                                    | 3.40              | 4.84  | -    | -    | 7.91  | -    | -     | -   | -   | 2.26  | -   | -    | -   | -   | -    | 1.22 | 0.80  |            |
| Maximum   | 10.87                        | 3.01                                    | 9.61              | 11.11 | -    | -    | 17.20 | -    | -     | -   | -   | 18.95 | -   | -    | -   | -   | -    | 6.55 | 6.66  |            |
| <b>Gastropods</b>   |                              |   |                   |       |      |      |       |      |       |     |     |       |     |      |     |     |      |      |       |            |
| <i>Conus gladiator</i> vs. ( <i>E. mus</i> , <i>E. tabidus</i> )  | 4.90                         | 1.20                                    | 4.10              | 9.20  |      |      |       |      |       |     |     |       |     |      |     |     |      |      | 0.60  | (164, 173) |
| <i>Conus ermineus</i> vs. <i>C. purpurascens</i>  | 3.75                         | 0.89                                    | 4.20              | 5.39  |      |      |       |      |       |     |     |       |     |      |     |     |      |      | 2.10  | (164, 174) |



|         |       |      |       |       |  |  |  |  |       |
|---------|-------|------|-------|-------|--|--|--|--|-------|
| Maximum | 40.12 | 4.52 | 27.50 | 40.12 |  |  |  |  | 26.64 |
|---------|-------|------|-------|-------|--|--|--|--|-------|

### Echinoids

|  |       |      |      |       |  |  |  |  |      |       |
|--|-------|------|------|-------|--|--|--|--|------|-------|
| -  |       |      |      |       |  |  |  |  |      |       |
| <i>Mellita</i>   |       |      |      |       |  |  |  |  |      |       |
| <i>quinquiesperforata</i> vs. ( <i>M. notabilis</i> , <i>M. kanakoffi</i> )  | 13.58 | 4.23 | 3.21 | 18.7  |  |  |  |  | 8.46 | (161) |
| ( <i>Mellita longifissa</i> , <i>M. grantii</i> ) vs. ( <i>M. isometra</i> , <i>M. tenuis</i> , <i>M. quinquiesperforata</i> ) | 24.47 | 4.48 | 5.46 | 39.76 |  |  |  |  | 9.18 | (161) |
| Median   | 19.03 | 4.36 | 4.34 | 29.23 |  |  |  |  | 8.82 |       |
| Minimum  | 13.58 | 4.23 | 3.21 | 18.70 |  |  |  |  | 8.46 |       |
| Maximum  | 24.47 | 4.48 | 5.46 | 39.76 |  |  |  |  | 9.18 |       |

|                        |              |             |              |              |  |  |  |  |  |  |
|------------------------|--------------|-------------|--------------|--------------|--|--|--|--|--|--|
| <b>Overall Median</b>  | <b>10.06</b> | <b>1.06</b> | <b>9.05</b>  | <b>12.72</b> |  |  |  |  |  |  |
| <b>Overall Minimum</b> | <b>2.10</b>  | <b>0.24</b> | <b>3.21</b>  | <b>4.84</b>  |  |  |  |  |  |  |
| <b>Overall Maximum</b> | <b>40.12</b> | <b>4.52</b> | <b>27.50</b> | <b>40.12</b> |  |  |  |  |  |  |

### No fossil calibration

#### Fishes

|  |      |  |      |      |      |      |      |      |      |                |
|--|------|--|------|------|------|------|------|------|------|----------------|
| <i>Diodon hystrix</i> vs. <i>D. hystrix</i>  | 0.20 |  | 0.60 | 0.00 | 0.00 |      |      |      |      | (3, 175)       |
| <i>Melichthys niger</i> vs. <i>M. niger</i>  | 0.40 |  | 0.20 | 0.60 | 0.40 |      |      |      |      | (3, 175)       |
| <i>Alutera scripta</i> vs. <i>A. scripta</i>   | 0.43 |  | 0.90 | 0.30 | 0.10 |      |      |      |      | (3, 175)       |
| <i>Diodon holacanthus</i> vs. <i>D. holacanthus</i>  | 0.45 |  |      | 0.30 | 0.60 |      |      |      |      | (3)            |
| <i>Mulloidichthys martinicus</i> vs. ( <i>M. dentatus</i> , <i>M. vanicolensis</i> , <i>M. mimicus</i> ) | 0.70 |  | 1.30 | 0.10 | 0.70 |      |      |      |      | (69)           |
| ( <i>Epinephelus clippertonensis</i> , <i>E. labriformis</i> ) vs. <i>E. adscensionis</i>                | 0.90 |  |      |      |      |      |      | 0.90 |      | (176)          |
| <i>Abudefduf concolor</i> vs. <i>A. taurus</i>   | 1.33 |  | 1.40 | 0.60 | 1.60 | 1.70 |      |      |      | (3, 177)       |
| <i>Anisotremus interruptus</i> vs. <i>A. surinamensis</i>  | 1.57 |  | 1.60 | 1.20 | 1.90 | 2.90 |      |      | 0.27 | (175, 178-180) |
| <i>Centropomus medius</i> vs. <i>C. pectinatus</i>   | 1.60 |  |      |      |      |      |      |      | 1.60 | (181)          |
| <i>Lutjanus cempachanus</i> vs. <i>L. peru</i>   | 1.77 |  | 1.56 |      | 1.97 |      | 1.78 |      |      | (182)          |
| <i>Cephalopholis panamensis</i> vs. <i>C. cruentatus</i>   | 1.90 |  |      |      |      |      |      | 1.20 | 2.60 | (176)          |
| <i>Lutjanus argentiventris</i> vs. <i>L. jocu</i>  | 2.15 |  | 0.88 |      | 2.98 |      | 2.60 |      |      | (182)          |
| <i>Lutjanus cyanopterus</i> vs. <i>L. novemfasciatus</i>   | 2.41 |  | 2.35 |      | 2.51 |      | 2.36 |      |      | (182)          |
| <i>Anisotremus taeniatus</i> vs. <i>A. virginicus</i>  | 3.11 |  | 4.40 | 3.70 | 3.80 | 3.23 |      |      | 0.43 | (175, 178-180) |
| <i>Abudefduf trashelii</i> vs. <i>A. saxatilis</i>   | 3.40 |  | 4.50 | 0.00 | 4.30 | 4.80 |      |      |      | (177, 183)     |
| <i>Sphyrna tiburo</i> vs. <i>S. tiburo</i>   | 3.50 |  |      |      | 3.50 |      |      |      |      | (184)          |

|   |       |       |       |       |       |      |       |       |       |                 |
|---|-------|-------|-------|-------|-------|------|-------|-------|-------|-----------------|
| <i>Gerres cinereus</i> vs. <i>G. cinereus</i>   | 3.90  | 5.10  | 2.70  | 3.90  |       |      |       |       |       | (3, 175, 185)   |
| <i>Holacanthus passer</i> vs. <i>H. ciliaris</i>  | 3.97  | 4.90  | 1.20  | 5.80  |       |      |       |       |       | (3, 175, 186)   |
| <i>Rypticus bicolor</i> vs. <i>R. saponaceus</i>  | 4.20  | 3.20  | 5.40  | 4.60  | 3.60  |      |       |       |       | (175, 185, 187) |
| <i>Lutjanus synagris</i> vs. <i>L. guttatus</i>   | 4.54  | 1.88  |       |       | 5.33  | 6.40 |       |       |       | (182)           |
| <i>Dermatolepis dermatolepis</i> vs. <i>D. inermis</i>  | 4.60  |       |       |       | 8.20  |      |       | 1.00  |       | (188)           |
| <i>Chaetodipterus zonatus</i> vs. <i>C. faber</i> ( <i>Tylosurus melanotus</i> , <i>T. pacificus</i> ) vs. ( <i>T. a. rafale</i> ( <i>T. a. imperialis</i> , <i>T. a. acus</i> ))   | 4.70  |       | 6.40  | 3.00  |       |      |       |       |       | (3, 185)        |
| <i>Haliichoeres nicholsi</i> vs. ( <i>H. bivittatus</i> , <i>H. garnoti</i> ) ( <i>H. radiatus</i> , <i>H. poeyii</i> ) ( <i>Hippocampus ingens</i> , <i>H. fisheri</i> ) vs. ( <i>H. reidi</i> , <i>H. algericus</i> ) ( <i>Alphistes immaculatus</i> , <i>A. multiguttatus</i> ) vs. <i>A. afer</i> | 4.77  |       | 4.20  | 5.70  | 4.40  |      |       |       |       | (189)           |
| <i>Scorpaena mystes</i> vs. <i>S. plumieri</i>  | 4.80  | 7.10  |       |       |       |      |       | 2.50  |       | (190)           |
| <i>Haeumulon steindachneri</i> vs. <i>H. steindachneri</i> ( <i>Scomberomorus sierra</i> , <i>S. concolor</i> ) vs. ( <i>S. regalis</i> , <i>S. brasiliensis</i> )  | 5.10  |       |       |       | 5.10  |      |       |       |       | (191)           |
| <i>Haliichoeres dispilus</i> vs. <i>H. pictus</i>   | 5.20  |       |       |       | 7.20  |      |       | 3.20  |       | (176, 188)      |
| <i>Lutjanus inermis</i> vs. <i>Ocyurus chrysurus</i>  | 5.50  | 5.50  |       |       |       |      |       |       |       | (175)           |
| <i>Paranthias colonus</i> vs. <i>P. furcifer</i>  | 5.83  | 6.58  |       |       | 5.08  |      |       |       |       | (192)           |
| <i>Ophioblennius macclurei</i> vs. <i>Ophioblennius steindachneri</i>   | 6.03  |       | 4.70  | 6.90  |       | 6.50 |       |       |       | (193)           |
| <i>Nicholsina denticulata</i> vs. <i>N. usta</i>  | 7.15  | 10.20 |       |       |       |      |       | 4.10  |       | (190)           |
| <i>Strongylura exilis</i> vs. ( <i>S. marina</i> , <i>S. sp</i> )   | 7.29  | 4.63  |       |       | 7.07  |      | 10.17 |       |       | (182)           |
| <i>Evorthodus minutus</i> vs. <i>E. lyricus</i>   | 7.83  | 4.80  | 19.60 | 6.70  |       |      |       | 0.20  |       | (3, 175, 176)   |
| <i>Centropomus rabalito</i> vs. <i>C. ensiferus</i>   | 11.23 | 12.40 | 5.60  | 12.70 | 14.20 |      |       |       |       | (3, 175, 194)   |
| <i>Strongylura scapularis</i> vs. <i>S. fluviatilis</i>   | 12.10 |       |       |       | 19.50 |      |       | 4.70  |       | (195-197)       |
| <i>Heteropriacanthus cruentatus</i> vs. <i>H. cruentatus</i>  | 12.42 |       | 20.00 | 14.60 | 14.20 |      |       | 5.10  | 8.20  | (189)           |
| <i>Acanthemblemaria betinensis</i> vs. <i>Acanthemblemaria exilispinus</i>  | 13.50 |       |       |       | 13.50 |      |       |       |       | (198)           |
| <i>Centropomus rabalito</i> vs. <i>C. ensiferus</i>   | 14.55 |       |       |       |       |      | 17.00 | 12.10 |       | (199)           |
| <i>Strongylura scapularis</i> vs. <i>S. fluviatilis</i>   | 15.18 |       | 15.60 | 16.60 | 21.60 |      |       | 10.90 | 11.20 | (189)           |
| <i>Heteropriacanthus cruentatus</i> vs. <i>H. cruentatus</i>  | 18.93 | 10.70 | 25.20 | 20.90 |       |      |       |       |       | (3, 175)        |
| <i>Acanthemblemaria betinensis</i> vs. <i>Acanthemblemaria exilispinus</i>  | 20.32 | 20.32 |       |       |       |      |       |       |       | (200)           |

## Crustaceans

|   |      |  |  |  |  |  |  |      |      |       |
|---|------|--|--|--|--|--|--|------|------|-------|
| <i>Chthamalus "mexicanus"</i> vs. <i>C. proteus</i> | 0.60 |  |  |  |  |  |  | 0.60 |      | (201) |
| <i>Petrolisthes armatus</i> vs. <i>P. armatus</i>   | 1.60 |  |  |  |  |  |  |      | 1.60 | (202) |
| <i>Callinectes toxotes</i> vs. <i>C. sapidus</i>    | 1.95 |  |  |  |  |  |  | 2.00 | 1.90 | (203) |

|   |       |       |       |            |
|---|-------|-------|-------|------------|
| <i>Euraphia eastropacensis</i> vs. <i>E. rhizophorae</i>  | 2.30  |       | 2.30  | (201)      |
| <i>Uca (Minuca) vocator</i> vs. <i>U. vocator</i>   | 2.40  |       | 2.40  | (204)      |
| <i>Callinectes arcuatus</i> vs. <i>C. ornatus</i>   | 2.55  |       | 3.00  | 2.10 (203) |
| <i>Petrolisthes galathinus</i> vs. <i>P. galathinus</i>   | 3.70  |       | 3.70  | (202)      |
| <i>Sesarma rhizophorae</i> vs. ( <i>S. reticulatum</i> , <i>S. sp. nr. reticulatum</i> , <i>S. curacaoense</i> )                  | 4.10  | 4.10  |       | (205)      |
| <i>Petrolisthes tonsorius</i> vs. <i>P. tonsorius</i>   | 4.90  |       | 4.90  | (202)      |
| <i>Alpheus antepenultimus</i> A vs. <i>A. chacei</i> ( <i>Sesarma sulcatum</i> , <i>S. aequatoriale</i> ) vs. <i>S. crassipes</i> | 5.40  | 5.40  |       | (206)      |
| <i>Alpheus rostratus</i> vs. <i>A. paracrinitus</i> spot  | 6.20  | 6.20  |       | (205)      |
| <i>Petrolisthes glasselli</i> vs. <i>P. rosariensis</i>   | 6.40  | 6.40  |       | (206)      |
| <i>Alpheus colombiensis</i> vs. <i>A. estuarensis</i>   | 6.70  |       | 6.70  | (202)      |
| <i>Alpheus websteri</i> vs. <i>A. websteri</i>  | 6.80  | 6.80  |       | (206)      |
| <i>Alpheus cylindricus</i> vs. <i>A. cylindricus</i>  | 7.30  | 7.30  |       | (206)      |
| <i>Synalpheus brevicarpus</i> vs. <i>S. brevicarpus</i>   | 8.70  | 8.70  |       | (207)      |
| <i>Alpheus canalis</i> -sp. B (blue) = <i>millsae</i> vs. <i>A. nuttingi</i>  | 8.70  | 8.70  |       | (206)      |
| <i>Alpheus floridanus</i> B' vs. <i>A. floridanus</i> B   | 9.40  | 9.40  |       | (206)      |
| <i>Alpheus panamensis</i> vs. <i>A. formosus</i> -sp. A   | 9.50  | 9.50  |       | (206)      |
| <i>Alpheus paracrinitus</i> no spot vs. <i>A. paracrinitus</i> no spot  | 9.50  | 9.50  |       | (206)      |
| <i>Synalpheus digueti</i> vs. <i>S. minus</i>   | 9.50  | 9.50  |       | (207)      |
| <i>Alpheus bouvieri</i> vs. <i>A. bouvieri</i>  | 11.00 | 11.00 |       | (206)      |
| <i>Xiphopenaeus riveti</i> vs. <i>X. sp. 2</i> ( <i>Petrolisthes haigae</i> , <i>P. hirtispinosus</i> ) vs. <i>P. marginatus</i>  | 11.10 | 11.10 |       | (208)      |
| <i>Alpheus malleator</i> vs. <i>A. malleator</i>  | 11.20 |       | 11.20 | (202)      |
| <i>Penaeus vannamei</i> vs. ( <i>P. duorarum</i> ( <i>P. paulensis</i> , <i>P. setiferus</i> ))                                   | 11.50 | 11.50 |       | (206)      |
| <i>Synalpheus fritzmuelleri</i> vs. <i>S. fritzmuelleri</i>   | 12.10 | 12.10 |       | (209)      |
| <i>Alpheus umbo</i> vs. <i>A. schmitti</i>  | 12.10 | 12.10 |       | (207)      |
| <i>Alpheus saxidomus</i> vs. <i>A. simus</i>  | 13.20 | 13.20 |       | (206)      |
| <i>Alpheus floridanus</i> A' vs. <i>A. floridanus</i> A   | 13.90 | 13.90 |       | (206)      |
| <i>Alpheus normanni</i> vs. <i>A. normanni</i> Brazil   | 15.10 | 15.10 |       | (206)      |
|   | 15.50 | 15.50 |       | (206)      |

