

## Additional file 9

### Supplementary results - evaluation of reference-guided scaffolding

**Table. Alignment summary for small scaffolds (scaffolds 14-31) of *Pristimantis euphronides*.** Each row reports one alignment between a small scaffold and a reference chromosome. Columns: Scaffold: *P. euphronides* scaffold identifier; Scaffold (kb): scaffold size in kilobases; Reference: comparison species (EleCoq = *Eleutherodactylus coqui*, EngPus = *Engystomops pustulosus*, DenEbr = *Dendropsophus ebraccatus*); Ref chr: target chromosome label (Ec1-Ec13, Ep1-Ep11, or De1-De15); Ref accession: NCBI RefSeq accession of the target chromosome; Aligned (kb): total aligned sequence in kilobases. All alignments computed with D-Genies v1.5.0 [1] (Minimap2 v2.28, default parameters). Genome assemblies: *E. coqui* haplotype 1 (GCF\_035609145.1); *E. pustulosus* maternal assembly (GCF\_040894005.1); *D. ebraccatus* paternal assembly (GCF\_027789765.1). For each scaffold, the three reference chromosomes per species with the highest total aligned base pairs are listed.

| Scaffold    | Scaffold (kb) | Reference | Ref chr | Ref accession | Aligned (kb) |
|-------------|---------------|-----------|---------|---------------|--------------|
| scaffold_14 | 55.1          | EleCoq    | Ec1     | NC_089837.1   | 1.7          |
| scaffold_14 | 55.1          | EleCoq    | Ec10    | NC_089846.1   | 0.1          |
| scaffold_14 | 55.1          | EngPus    | Ep4     | NC_092414.1   | 7.6          |
| scaffold_14 | 55.1          | EngPus    | Ep11    | NC_092421.1   | 1.5          |
| scaffold_14 | 55.1          | EngPus    | Ep5     | NC_092415.1   | 0.7          |
| scaffold_14 | 55.1          | DenEbr    | De1     | NC_091454.1   | 5.5          |
| scaffold_14 | 55.1          | DenEbr    | De3     | NC_091456.1   | 0.9          |
| scaffold_14 | 55.1          | DenEbr    | De7     | NC_091460.1   | 0.6          |
| scaffold_15 | 82.9          | EleCoq    | Ec7     | NC_089843.1   | 1.1          |
| scaffold_15 | 82.9          | EleCoq    | Ec4     | NC_089840.1   | 0.7          |
| scaffold_15 | 82.9          | EleCoq    | Ec12    | NC_089848.1   | 0.5          |
| scaffold_15 | 82.9          | EngPus    | Ep2     | NC_092412.1   | 1.9          |
| scaffold_15 | 82.9          | EngPus    | Ep3     | NC_092413.1   | 1.3          |
| scaffold_15 | 82.9          | EngPus    | Ep8     | NC_092418.1   | 0.7          |
| scaffold_15 | 82.9          | DenEbr    | De5     | NC_091458.1   | 19.2         |
| scaffold_15 | 82.9          | DenEbr    | De3     | NC_091456.1   | 1.0          |
| scaffold_15 | 82.9          | DenEbr    | De6     | NC_091459.1   | 0.7          |

|             |       |        |      |             |      |
|-------------|-------|--------|------|-------------|------|
| scaffold_16 | 19.0  | DenEbr | De12 | NC_091465.1 | 0.7  |
| scaffold_17 | 136.8 | EleCoq | Ec4  | NC_089840.1 | 17.4 |
| scaffold_17 | 136.8 | EleCoq | Ec3  | NC_089839.1 | 2.6  |
| scaffold_17 | 136.8 | EleCoq | Ec1  | NC_089837.1 | 2.5  |
| scaffold_17 | 136.8 | EngPus | Ep5  | NC_092415.1 | 24.2 |
| scaffold_17 | 136.8 | EngPus | Ep4  | NC_092414.1 | 9.2  |
| scaffold_17 | 136.8 | EngPus | Ep1  | NC_092411.1 | 7.4  |
| scaffold_17 | 136.8 | DenEbr | De4  | NC_091457.1 | 23.7 |
| scaffold_17 | 136.8 | DenEbr | De2  | NC_091455.1 | 5.2  |
| scaffold_17 | 136.8 | DenEbr | De1  | NC_091454.1 | 4.9  |
| scaffold_18 | 81.0  | EleCoq | Ec5  | NC_089841.1 | 22.6 |
| scaffold_18 | 81.0  | EleCoq | Ec2  | NC_089838.1 | 13.7 |
| scaffold_18 | 81.0  | EleCoq | Ec1  | NC_089837.1 | 11.8 |
| scaffold_18 | 81.0  | EngPus | Ep1  | NC_092411.1 | 15.2 |
| scaffold_18 | 81.0  | EngPus | Ep2  | NC_092412.1 | 4.1  |
| scaffold_18 | 81.0  | EngPus | Ep7  | NC_092417.1 | 3.1  |
| scaffold_18 | 81.0  | DenEbr | De10 | NC_091463.1 | 8.6  |
| scaffold_18 | 81.0  | DenEbr | De2  | NC_091455.1 | 3.4  |
| scaffold_18 | 81.0  | DenEbr | De8  | NC_091461.1 | 2.4  |
| scaffold_19 | 10.2  | EleCoq | Ec1  | NC_089837.1 | 0.8  |
| scaffold_19 | 10.2  | EleCoq | Ec12 | NC_089848.1 | 0.4  |
| scaffold_19 | 10.2  | EngPus | Ep3  | NC_092413.1 | 0.8  |
| scaffold_19 | 10.2  | DenEbr | De6  | NC_091459.1 | 1.8  |
| scaffold_19 | 10.2  | DenEbr | De12 | NC_091465.1 | 1.0  |
| scaffold_20 | 67.5  | EleCoq | Ec6  | NC_089842.1 | 2.6  |
| scaffold_20 | 67.5  | EleCoq | Ec8  | NC_089844.1 | 0.9  |
| scaffold_20 | 67.5  | EleCoq | Ec1  | NC_089837.1 | 0.1  |
| scaffold_20 | 67.5  | EngPus | Ep3  | NC_092413.1 | 6.5  |
| scaffold_20 | 67.5  | EngPus | Ep10 | NC_092420.1 | 0.8  |
| scaffold_20 | 67.5  | EngPus | Ep8  | NC_092418.1 | 0.3  |
| scaffold_20 | 67.5  | DenEbr | De6  | NC_091459.1 | 10.7 |
| scaffold_20 | 67.5  | DenEbr | De13 | NC_091466.1 | 3.4  |
| scaffold_20 | 67.5  | DenEbr | De15 | NC_091468.1 | 0.3  |
| scaffold_21 | 100.5 | EleCoq | Ec13 | NC_089849.1 | 7.9  |
| scaffold_21 | 100.5 | EleCoq | Ec1  | NC_089837.1 | 4.4  |
| scaffold_21 | 100.5 | EleCoq | Ec2  | NC_089838.1 | 4.3  |
| scaffold_21 | 100.5 | EngPus | Ep3  | NC_092413.1 | 7.5  |
| scaffold_21 | 100.5 | EngPus | Ep4  | NC_092414.1 | 4.3  |
| scaffold_21 | 100.5 | EngPus | Ep1  | NC_092411.1 | 1.2  |
| scaffold_21 | 100.5 | DenEbr | De13 | NC_091466.1 | 7.5  |
| scaffold_21 | 100.5 | DenEbr | De2  | NC_091455.1 | 4.5  |
| scaffold_21 | 100.5 | DenEbr | De1  | NC_091454.1 | 3.6  |

|             |       |        |      |             |      |
|-------------|-------|--------|------|-------------|------|
| scaffold_22 | 55.9  | EleCoq | Ec8  | NC_089844.1 | 2.8  |
| scaffold_22 | 55.9  | EngPus | Ep3  | NC_092413.1 | 1.9  |
| scaffold_22 | 55.9  | DenEbr | De15 | NC_091468.1 | 15.3 |
| scaffold_22 | 55.9  | DenEbr | De6  | NC_091459.1 | 1.6  |
| scaffold_22 | 55.9  | DenEbr | De11 | NC_091464.1 | 0.7  |
| scaffold_24 | 350.9 | EleCoq | Ec1  | NC_089837.1 | 5.7  |
| scaffold_24 | 350.9 | EleCoq | Ec6  | NC_089842.1 | 4.7  |
| scaffold_24 | 350.9 | EleCoq | Ec2  | NC_089838.1 | 1.4  |
| scaffold_24 | 350.9 | EngPus | Ep5  | NC_092415.1 | 26.7 |
| scaffold_24 | 350.9 | EngPus | Ep7  | NC_092417.1 | 4.0  |
| scaffold_24 | 350.9 | EngPus | Ep6  | NC_092416.1 | 1.1  |
| scaffold_24 | 350.9 | DenEbr | De4  | NC_091457.1 | 22.1 |
| scaffold_24 | 350.9 | DenEbr | De13 | NC_091466.1 | 5.2  |
| scaffold_24 | 350.9 | DenEbr | De6  | NC_091459.1 | 2.2  |
| scaffold_25 | 131.5 | EleCoq | Ec7  | NC_089843.1 | 4.0  |
| scaffold_25 | 131.5 | EleCoq | Ec8  | NC_089844.1 | 1.1  |
| scaffold_25 | 131.5 | EngPus | Ep1  | NC_092411.1 | 17.8 |
| scaffold_25 | 131.5 | DenEbr | De7  | NC_091460.1 | 12.4 |
| scaffold_25 | 131.5 | DenEbr | De5  | NC_091458.1 | 3.3  |
| scaffold_25 | 131.5 | DenEbr | De12 | NC_091465.1 | 0.6  |
| scaffold_26 | 139.8 | EleCoq | Ec1  | NC_089837.1 | 55.8 |
| scaffold_26 | 139.8 | EleCoq | Ec3  | NC_089839.1 | 17.8 |
| scaffold_26 | 139.8 | EleCoq | Ec5  | NC_089841.1 | 3.7  |
| scaffold_26 | 139.8 | EngPus | Ep6  | NC_092416.1 | 25.4 |
| scaffold_26 | 139.8 | EngPus | Ep4  | NC_092414.1 | 7.5  |
| scaffold_26 | 139.8 | EngPus | Ep9  | NC_092419.1 | 1.2  |
| scaffold_26 | 139.8 | DenEbr | De8  | NC_091461.1 | 14.3 |
| scaffold_26 | 139.8 | DenEbr | De1  | NC_091454.1 | 4.1  |
| scaffold_26 | 139.8 | DenEbr | De4  | NC_091457.1 | 0.7  |
| scaffold_27 | 302.4 | EleCoq | Ec2  | NC_089838.1 | 13.6 |
| scaffold_27 | 302.4 | EleCoq | Ec4  | NC_089840.1 | 5.7  |
| scaffold_27 | 302.4 | EleCoq | Ec13 | NC_089849.1 | 4.5  |
| scaffold_27 | 302.4 | EngPus | Ep5  | NC_092415.1 | 22.2 |
| scaffold_27 | 302.4 | EngPus | Ep7  | NC_092417.1 | 11.2 |
| scaffold_27 | 302.4 | EngPus | Ep8  | NC_092418.1 | 6.0  |
| scaffold_27 | 302.4 | DenEbr | De4  | NC_091457.1 | 23.7 |
| scaffold_27 | 302.4 | DenEbr | De9  | NC_091462.1 | 11.6 |
| scaffold_27 | 302.4 | DenEbr | De1  | NC_091454.1 | 11.3 |
| scaffold_28 | 78.7  | EleCoq | Ec1  | NC_089837.1 | 14.5 |
| scaffold_28 | 78.7  | EleCoq | Ec11 | NC_089847.1 | 6.2  |
| scaffold_28 | 78.7  | EleCoq | Ec2  | NC_089838.1 | 3.6  |
| scaffold_28 | 78.7  | EngPus | Ep11 | NC_092421.1 | 1.6  |

|             |       |        |      |             |      |
|-------------|-------|--------|------|-------------|------|
| scaffold_28 | 78.7  | EngPus | Ep4  | NC_092414.1 | 0.9  |
| scaffold_28 | 78.7  | DenEbr | De1  | NC_091454.1 | 37.6 |
| scaffold_28 | 78.7  | DenEbr | De10 | NC_091463.1 | 5.0  |
| scaffold_28 | 78.7  | DenEbr | De7  | NC_091460.1 | 4.9  |
| scaffold_29 | 40.9  | EleCoq | Ec2  | NC_089838.1 | 3.1  |
| scaffold_29 | 40.9  | EleCoq | Ec13 | NC_089849.1 | 0.4  |
| scaffold_29 | 40.9  | EleCoq | Ec1  | NC_089837.1 | 0.2  |
| scaffold_29 | 40.9  | EngPus | Ep11 | NC_092421.1 | 0.4  |
| scaffold_29 | 40.9  | DenEbr | De1  | NC_091454.1 | 8.5  |
| scaffold_29 | 40.9  | DenEbr | De4  | NC_091457.1 | 1.0  |
| scaffold_30 | 216.9 | EleCoq | Ec11 | NC_089847.1 | 5.3  |
| scaffold_30 | 216.9 | EleCoq | Ec13 | NC_089849.1 | 5.2  |
| scaffold_30 | 216.9 | EleCoq | Ec3  | NC_089839.1 | 5.2  |
| scaffold_30 | 216.9 | EngPus | Ep8  | NC_092418.1 | 12.0 |
| scaffold_30 | 216.9 | EngPus | Ep4  | NC_092414.1 | 4.1  |
| scaffold_30 | 216.9 | EngPus | Ep6  | NC_092416.1 | 1.0  |
| scaffold_30 | 216.9 | DenEbr | De9  | NC_091462.1 | 29.0 |
| scaffold_30 | 216.9 | DenEbr | De1  | NC_091454.1 | 12.0 |
| scaffold_30 | 216.9 | DenEbr | De14 | NC_091467.1 | 11.8 |
| scaffold_31 | 33.7  | EleCoq | Ec11 | NC_089847.1 | 13.0 |
| scaffold_31 | 33.7  | EleCoq | Ec5  | NC_089841.1 | 5.5  |
| scaffold_31 | 33.7  | EleCoq | Ec13 | NC_089849.1 | 2.0  |
| scaffold_31 | 33.7  | EngPus | Ep1  | NC_092411.1 | 10.3 |
| scaffold_31 | 33.7  | EngPus | Ep2  | NC_092412.1 | 4.7  |
| scaffold_31 | 33.7  | EngPus | Ep4  | NC_092414.1 | 4.5  |
| scaffold_31 | 33.7  | DenEbr | De8  | NC_091461.1 | 2.3  |
| scaffold_31 | 33.7  | DenEbr | De3  | NC_091456.1 | 1.4  |
| scaffold_31 | 33.7  | DenEbr | De15 | NC_091468.1 | 0.3  |

## Reference

1. Cabanettes F, Klopp C. D-GENIES: dot plot large genomes in an interactive, efficient and simple way. PeerJ. 2018 Jun 4;6:e4958.