Supplementary Material 1

# Supplementary Data

*List of scientific collections assessed:*

CNRS/AF - Personal Collection Antonie Fouguet (Cayenne, French Guiana)

INPA/HT - Herpetology Collection at the Instituto Nacional de Pesquisas da Amazônia (Manaus, Brazil)

MTR – Miguel T. Rodrigues collection at the Universidade Federal de São Paulo (São Paulo, Brazil)

PUCRS - Pontifícia Universidade Católica do Rio Grande do Sul (Porto Alegre, Brazil)

UFBA - Herpetology Collection of Universidade Federal da Bahia (Salvador, Brazil)

UFES - Herpetology Collection of Universidade Federal do Espírito Santo (Vitória, Brazil)

UFRN - Herpetology Collection of Universidade Federal do Rio Grande do Norte (Natal, Brazil)

UNESP - Herpetology Collection of Universidade Estadual Paulista Júlio de Mesquita Filho (Rio

Claro, Brazil)

# Supplementary Figures and Tables

**Supplementary Table S1.** Sampled specimens used in next-generation sequencing, with taxon, voucher number and locality of origin. \*these samples were discarded from the analyses.

| **Taxon** | **Voucher** | **Latitude** | **Longitude** | **Locality** | **State** | **Country** |
| --- | --- | --- | --- | --- | --- | --- |
| *D. elegans* | H-568 | -23.8064 | -46.0590 | Bertioga | São Paulo | Brazil |
| *D. elegans* | H-579 | -23.8064 | -46.0590 | Bertioga | São Paulo | Brazil |
| *D. elegans* | JC804 | -20.3633 | -43.3702 | Mariana | Minas Gerais | Brazil |
| *D. elegans* | JFT1035 | -13.3537 | -39.0879 | Valença | Bahia | Brazil |
| *D. elegans* | MRT5820 | -15.1138 | -39.5382 | Serra do Teimoso, Jussari | Bahia | Brazil |
| *D. elegans* | MRT5879 | -15.1138 | -39.5382 | Serra do Teimoso, Jussari | Bahia | Brazil |
| *D. elegans* | MTR12063 | -19.3616 | -40.0878 | Linhares, Reserva da Companhia Vale do Rio Doce | Espírito Santo | Brazil |
| *D. elegans* | MTR12145 | -19.3616 | -40.0878 | Linhares, Reserva da Companhia Vale do Rio Doce | Espírito Santo | Brazil |
| *D. elegans* | MTR12594 | -20.5201 | -41.7176 | Parque Nacional do Caparaó, Santa Maria | Espírito Santo | Brazil |
| *D. elegans* | MTR12606 | -20.5201 | -41.7176 | Parque Nacional do Caparaó, Santa Maria | Espírito Santo | Brazil |
| *D. elegans* | MTR17020 | -22.1972 | -48.7754 | E.B. Boracéia | São Paulo | Brazil |
| *D. elegans* | MTR17021 | -22.1972 | -48.7754 | E.B. Boracéia | São Paulo | Brazil |
| *D. elegans* | MTR17218 | -16.4333 | -40.7832 | Reserva Biológica Mata Escura, Jequitinhonha | Minas Gerais | Brazil |
| *D. elegans* | MTR17562 | -19.7097 | -42.7345 | Parque Estadual do Rio Doce, Marliéria | Minas Gerais | Brazil |
| *D. elegans* | MTR22883 | -22.5991 | -43.2230 | Taquara, Duque de Caxias | Rio de Janeiro | Brazil |
| *D. elegans* | UFBA7872 | -12.5151 | -38.0355 | Mata de São João, Reserva Camurujipe, Ponto 1 | Bahia | Brazil |
| *D. elegans* | UFBA7873 | -12.5151 | -38.0355 | Mata de São João, Reserva Camurujipe | Bahia | Brazil |
| *D. elegans* | UFRN3666 | -20.7594 | -42.8618 | Mata da Biologia - campus UFV, Viçosa | Minas Gerais | Brazil |
| *D. elegans* | UFRN9039 | -9.7625 | -36.1328 | São Miguel dos Campos | Alagoas | Brazil |
| *D. elegans* | UFRN9158 | -11.2651 | -37.4215 | Estância | Sergipe | Brazil |
| *D. elegans* | UFRN9700 | -12.9711 | -39.3949 | Varzedo | Bahia | Brazil |
| *D. elegans* | UFRN9705 | -12.9711 | -39.3949 | Varzedo | Bahia | Brazil |
| *D. elegans* | UNESP17704 | -24.7098 | -47.5562 | Iguape | São Paulo | Brazil |
| *D. elegans* | UNESP17710 | -24.7098 | -47.5562 | Iguape | São Paulo | Brazil |
| *D. elegans* | UNESP21300 | -25.3988 | -48.8492 | São João da Graciosa, Morretes | Paraná | Brazil |
| *D. elegans* | UNESP23214 | -25.9155 | -48.9134 | Fazenda Creminário, Serra do Araraquara, Guaratuba | Paraná | Brazil |
| *D. elegans* | UNESP27568 | -20.7644 | -42.8695 | Mata da Biologia - campus UFV, Viçosa | Minas Gerais | Brazil |
| *D. elegans* | UNESP6305 | -24.5845 | -48.6009 | PETAR - Núcleo Ouro Grosso, Iporanga | São Paulo | Brazil |
| *D. elegans* | UNESP6306 | -24.5845 | -48.6009 | PETAR - Núcleo Ouro Grosso, Iporanga | São Paulo | Brazil |
| *D. anceps* | MTR12377 | -19.3951 | -40.0647 | Linhares, Reserva da Companhia Vale do Rio Doce | Espírito Santo | Brazil |
| *D. anceps* | MTR12375 | -19.3951 | -40.0647 | Linhares, Reserva da Companhia Vale do Rio Doce | Espírito Santo | Brazil |
| *D. anceps* | MTR17494 | -19.7398 | -42.6432 | Marliéria, entorno PE Rio Doce | Minas Gerais | Brazil |
| *D. anceps* | MTR17495 | -19.7398 | -42.6432 | Marliéria, entorno PE Rio Doce | Minas Gerais | Brazil |
| *D. leucophyllatus* | AF0606 | 5.3735 | -53.0976 | Trou Poisson | Mare | French Guiana |
| *D. leucophyllatus* | AF1096 | 3.9040 | -51.7723 | Oiapoque | Amapá | Brazil |
| *D. minutus* | HT4308 | -2.6762 | -54.9355 | Belterra Poção | Pará | Brazil |
| *D. minutus* | HT4309 | -2.6762 | -54.9355 | Belterra Poção | Pará | Brazil |
| *\*D. elegans* | MTR 17110 | -16.4378 | -41.1043 | Jequitinhonha, Estrada para Itaobim | Minas Gerais | Brazil |
| *\*D. elegans* | JFT1034 | -13.3537 | -39.0879 | Valença | Bahia | Brazil |
| *\*D. elegans* | MTR17217 | -16.4333 | -40.7832 | Reserva Biológica Mata Escura, Jequitinhonha | Minas Gerais | Brazil |
| *\*D. elegans* | MTR22884 | -22.5991 | -43.2230 | Taquara, Duque de Caxias | Rio de Janeiro | Brazil |
| *\*D. elegans* | UFBA7871 | -12.515 | -38.035 | Mata de São João, Reserva Camurujipe | Bahia | Brazil |
| *\*D. elegans* | UNESP38104 | -20.0739 | -43.4065 | Serra do Caraça, Catas Altas | Minas Gerais | Brazil |
| *\*D. elegans* | UFBA7867 | -12.511 | -38.042 | Mata de São João, Reserva Camurujipe | Bahia | Brazil |

# Summaries of genomic data

**Supplementary Table S2.** Processing information and ipyRAD summary statistics for specimens sequenced. Raw reads refer to the total number of reads produced during Illumina sequencing; reads passed filter represent post-processing number of reads that remained after filtering for quality, adaptor contamination, ambiguous barcodes, and mitochondrial sequences; total clusters are the number of homologous sequences clusters created with the post-processing reads; mean depth is the mean depth of coverage of a cluster. Heterozygosity\_estimated (H) and error-estimated (E) were estimated across clusters, and consensus sequences were created for each cluster. The missing data for each individual of *Dendropsophus elegans* is also presented (secondary dataset). Variable and invariable DNA sites were summed across all loci (Total sites), and the percentage of polymorphic sites (% poly) is presented. Consensus sequences were clustered across specimens, and loci that passed filtering parameters were included in the final data matrix (Final loci). \*Discarded specimens with low number of reads (<500,000).

| **Taxon** | **raw reads** | **reads passed filter** | **total clusters** | **clusters\_hidepth** | **H** | **E** | **reads\_consens** | **loci\_in\_assembly** | **Missing data** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Danc\_MRT12377\_LiEsBr | 1281593 | 1280936 | 148019 | 61882 | 0.009721 | 0.004339 | 56895 | 3089 | - |
| Danc\_MTR12375\_LiEsBr | 1293414 | 1292706 | 151994 | 60438 | 0.009728 | 0.004677 | 55478 | 3043 | - |
| Danc\_MTR17494\_MaMgBr | 1592323 | 1591430 | 161731 | 66211 | 0.009204 | 0.004116 | 61524 | 3199 | - |
| Danc\_MTR17495\_MaMgBr | 1393824 | 1393029 | 160565 | 63744 | 0.008771 | 0.004475 | 58883 | 3142 | - |
| Deleg\_H-568\_BeSpBr | 1551919 | 1550954 | 167148 | 65825 | 0.008211 | 0.004535 | 60452 | 24213 | 0.007363 |
| Deleg\_H-579\_BeSpBr | 2035179 | 2033877 | 186003 | 74374 | 0.008739 | 0.004673 | 68043 | 25046 | 0.007154 |
| Deleg\_JC804\_MaMgBr | 1430618 | 1429723 | 174383 | 67066 | 0.010963 | 0.004518 | 60335 | 22818 | 0.008668 |
| \*Deleg\_JFT1034\_VaBaBr | 144979 | 144890 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| Deleg\_JFT1035\_VaBaBr | 1553619 | 1552666 | 172462 | 65713 | 0.010034 | 0.004767 | 59847 | 23108 | 0.00731 |
| Deleg\_MRT5820\_StJuBaBr | 875037 | 874471 | 161211 | 48723 | 0.013886 | 0.004897 | 42162 | 18167 | 0.01572 |
| Deleg\_MRT5879\_StJuBaBr | 1265900 | 1265114 | 184740 | 65901 | 0.012805 | 0.004505 | 58411 | 22387 | 0.01008 |
| Deleg\_MTR12063\_LiEsBr | 1084153 | 1083403 | 163250 | 56977 | 0.01299 | 0.004359 | 50339 | 20326 | 0.01368 |
| Deleg\_MTR12145\_LiEsBr | 1358362 | 1357484 | 191497 | 67183 | 0.012721 | 0.00488 | 59327 | 22590 | 0.009503 |
| Deleg\_MTR12594\_CaSmEsBr | 1309641 | 1308847 | 180125 | 67693 | 0.012437 | 0.004383 | 60235 | 23271 | 0.01018 |
| Deleg\_MTR12606\_CaSmEsBr | 933543 | 932922 | 154362 | 54196 | 0.013226 | 0.004595 | 47423 | 20332 | 0.01546 |
| Deleg\_MTR17020\_BoSpBr | 1332677 | 1331769 | 173282 | 63089 | 0.008921 | 0.004751 | 57304 | 23521 | 0.007832 |
| Deleg\_MTR17021\_BoSpBr | 1145967 | 1145249 | 177882 | 59198 | 0.00952 | 0.004909 | 53239 | 22461 | 0.01086 |
| \*Deleg\_MTR17110\_JeMgBr | 959 | 958 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| \*Deleg\_MTR17217\_JeMgBr | 73867 | 73818 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| Deleg\_MTR17218\_JeMgBr | 1178425 | 1177540 | 164005 | 60185 | 0.01254 | 0.004771 | 53471 | 21127 | 0.01023 |
| Deleg\_MTR17562\_MaMgBr | 1398627 | 1397728 | 184899 | 69189 | 0.011693 | 0.004724 | 61735 | 22919 | 0.009869 |
| Deleg\_MTR22883\_DuRjBr | 1025563 | 1024806 | 143182 | 53239 | 0.010754 | 0.004694 | 47827 | 20969 | 0.0118 |
| \*Deleg\_MTR22884\_DuRjBr | 122586 | 122517 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| \*Deleg\_UFBA7867\_SjBaBr | 402196 | 401946 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| \*Deleg\_UFBA7871\_SjBaBr | 481534 | 481241 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| Deleg\_UFBA7872\_MsBar | 551291 | 551097 | 114035 | 30531 | 0.011099 | 0.002628 | 27902 | 12220 | 0.02151 |
| Deleg\_UFBA7873\_MsBar | 973060 | 972711 | 126524 | 52510 | 0.009556 | 0.001868 | 48999 | 19497 | 0.009242 |
| Deleg\_UFRN3666\_VcMgBr | 1774055 | 1772886 | 220297 | 77534 | 0.011786 | 0.004459 | 69569 | 24695 | 0.008146 |
| Deleg\_UFRN9039\_SaoAlBr | 2304782 | 2303325 | 190177 | 73207 | 0.008891 | 0.004443 | 67649 | 23045 | 0.008616 |
| Deleg\_UFRN9158\_EtSeBr | 2376309 | 2374871 | 188322 | 76074 | 0.009259 | 0.00435 | 70125 | 24275 | 0.006527 |
| Deleg\_UFRN9700\_VaBaBr | 2395613 | 2394162 | 191555 | 78737 | 0.010743 | 0.004502 | 71715 | 24600 | 0.006423 |
| Deleg\_UFRN9705\_VaBaBr | 1838441 | 1837356 | 178541 | 74155 | 0.010263 | 0.004323 | 67893 | 24037 | 0.006214 |
| Deleg\_UNESP17704\_IgSpBr | 1409771 | 1408864 | 166153 | 60272 | 0.006472 | 0.004332 | 56194 | 24799 | 0.007415 |
| Deleg\_UNESP17710\_IgSpBr | 2088881 | 2087642 | 176393 | 72314 | 0.006128 | 0.004321 | 67548 | 26104 | 0.006214 |
| Deleg\_UNESP21300\_MoPrBr | 1441622 | 1440686 | 177472 | 61006 | 0.006012 | 0.004458 | 56967 | 25064 | 0.006997 |
| Deleg\_UNESP23214\_GuPrBr | 930197 | 929481 | 143011 | 49741 | 0.005159 | 0.004923 | 46165 | 21411 | 0.007676 |
| Deleg\_UNESP27568\_VcMgBr | 2044775 | 2043304 | 218411 | 76920 | 0.01209 | 0.004519 | 68796 | 24762 | 0.006684 |
| \*Deleg\_UNESP38104\_CaMgBr | 1962 | 1962 | 0 | 0 | NaN | NaN | 0 | NaN | - |
| Deleg\_UNESP6305\_IpSpBr | 1500600 | 1499655 | 185788 | 66540 | 0.005965 | 0.004489 | 62018 | 25176 | 0.006945 |
| Deleg\_UNESP6306\_IpSpBr | 1467774 | 1466919 | 187791 | 65000 | 0.005594 | 0.004688 | 60612 | 25092 | 0.00731 |
| Dleuco\_AF0606\_TpMaFg | 1453823 | 1452826 | 199432 | 70748 | 0.01225 | 0.005683 | 62137 | 4000 | - |
| Dleuco\_AF1096\_OiApBr | 1504734 | 1503718 | 193274 | 72536 | 0.012291 | 0.005497 | 63979 | 3971 | - |
| Dmin\_HT4308\_BtPaBr | 914472 | 913992 | 143690 | 50658 | 0.007644 | 0.004896 | 46745 | 2348 | - |
| Dmin\_HT4309\_BtPaBr | 1098224 | 1097649 | 163875 | 57219 | 0.007647 | 0.004385 | 53234 | 2516 | - |

**Supplementary Figure S1.** Results from the original ipyRAD output file. A systematic increase in the frequency of variable site (SNP) can be found close to the end among the aligned sequences (around position 130 [a red solid line])

Chart, histogram

Description automatically generated

# Methods

**Supplementary Figure S2.** Maximum likelihood tree based on a concatenated matrix of 3,852,884 loci (all the sequences dataset including missing data) analyzed in RaxML v.8, using the GTRCAT model for all the sampled individuals. Clades colors correspond to the population genetic analysis results. Values above the branches indicate clade support, based on 100 bootstrap pseudoreplicates.

A picture containing arrow

Description automatically generated

**Supplementary Figure S3.** Diagnostic plot ofDelta K and the Likelihood K from STRUCTURE using 5,000 unlinked SNPs randomly selected with no outgroups. Ten replicated analyses were ran over a range of K from 1 to 4.

**Chart, scatter chart

Description automatically generated**

**Supplementary Figure S4.** Diagnostic plots of K from Structure Selector (Li and Liu, 2018), using Puechmaille's method (2016) for 5,000 unlinked SNPs randomly selected with no outgroups. Ten replicated analyses were ran over a range of K from 1 to 4.

**Supplementary Table S3.** Pairwise *Ψ* statistics of *Dendropsophus elegans* populations and localities.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **North\_1** | **North\_2** | **North\_3** | **North\_4** | **North\_5** |
| North\_1 | - | 0.0442 | 0.1011 | 0.0921 | 0.3351 |
| North\_2 | 0.0442 | - | 0.0529 | 0.0426 | 0.2609 |
| North\_3 | 0.1011 | 0.0529 | - | 0.0003 | 0.2191 |
| North\_4 | 0.0921 | 0.0426 | 0.0003 | - | 0.2139 |
| North\_5 | 0.3351 | 0.2609 | 0.2191 | 0.2139 | - |
|  | **South\_1** | **South\_2** | **South\_3** | **South\_4** |  |
| South\_1 | - | -0.2265 | -0.5516 | -0.1771 | - |
| South\_2 | -0.2265 | - | -0.3593 | -0.1031 | - |
| South\_3 | -0.5516 | -0.3593 | - | 0.1838 | - |
| South\_4 | -0.1771 | -0.1031 | 0.1838 | - | - |

Regions and samples: North\_1 (Deleg\_UFBA7872\_MsBar, Deleg\_UFBA7873\_MsBar); North\_2 (Deleg\_JFT1035\_VaBaBr, Deleg\_UFRN9700\_VaBaBr; Deleg\_UFRN9705\_VaBaBr), North\_3 (Deleg\_MRT5820\_StJuBaBr, Deleg\_MRT5879\_StJuBaBr); North\_4 (Deleg\_MTR12063\_LiEsBr, Deleg\_MTR12145\_LiEsBr), North\_5 (Deleg\_MTR17562\_MaMgBr, Deleg\_JC804\_MaMgBr); South\_1 (Deleg\_H-568\_BeSpBr, Deleg\_H-579\_BeSpBr); South\_2 (Deleg\_MTR17020\_BoSpBr, Deleg\_MTR17021\_BoSpBr); South\_3 (Deleg\_UNESP17704\_IgSpBr; Deleg\_UNESP17710\_IgSpBr); and South\_4 (Deleg\_UNESP6305\_IpSpBr; Deleg\_UNESP6306\_IpSpBr; Deleg\_UNESP21300\_MoPrBr; Deleg\_UNESP23214\_GuPrBr).

**References**

Li, Y.L. and Liu, J.X. (2018). StructureSelector: A web based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour. 18:176–177. doi: 10.1111/1755-0998.12719

Puechmaille S.J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour. 16, 608–627. doi:10.1111/1755-0998.12512

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