*Molecular Phylogenetics and Evolution*

**SUPPLEMENTARY MATERIAL**

**Phylogeography of the arid-adapted Malagasy bullfrog, *Laliostoma labrosum*, influenced by past connectivity and habitat stability**

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**Table S1**. List of field numbers of ethanol preserved tissue samples with corresponding geographical coordinates and localities. Accession numbers for gene sequences amplified from each specimen are listed, as well as mitochondrial haplotype designation. N – north; W1 – west 1; W2 – west 2; W3 – west 3; Sc1 – south-central 1; Sc2 – south-central 2; Sw – southwest.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| field number | locality | mtDNA hap | 16S rRNA | *cytb* | *cox1* | 12S rRNA | *bdnf* | *pomc* | *rag1* |
| FGMV2002.2078 | Ambalavao | W3 | KR337867 | KR479587 | – | – | KR479709 | KR809612 | KR479759 |
| FGZC19 | Ambalavao | W3 | AY848009 | KR479588 | – | – | KR479645 | KR809617 | KR479765 |
| FGZC20 | Ambalavao | W3 | AY848010 | KR479589 | – | – | KR479695 | KR809618 | KR479766 |
| HER2791 | Ampananira | W3 | KR337902 | KR479603 | – | – | KR479723 | KR809647 | KR479791 |
| HER2649 | Ampasibe | W2 | KR337897 | KR479598 | – | – | KR479743 | KR809642 | KR479789 |
| HER2680 | Ampasibe | W3 | KR337898 | KR479599 | – | – | KR479744 | KR809643 | – |
| FGZC2538 | Andohahela | SC1 | KR337876 | KR479592 | – | – | KR479642 | KR809621 | KR479769 |
| HER3024 | Analamavo Forest | W1 | KR337904 | KR479605 | – | – | KR479715 | KR809649 | KR479792 |
| HER3136 | Analamavo Forest | W2 | KR337905 | KR479606 | – | – | KR479719 | KR809650 | KR479793 |
| MPFC0289 | Ankarafantsika | W1 | KR337941 | KR348299 | KR337847 | KR337834 | KR479663 | KR809678 | KR479829 |
| ZCMV5617 | Ankarafantsika | W1 | KR337952 | KR348298 | – | – | – | – | KR479839 |
| ZCMV5618 | Ankarafantsika | W1 | KR337953 | KR348300 | – | – | KR479664 | KR809684 | KR479840 |
| ZCMV5619 | Ankarafantsika | W1 | KR337954 | KR348301 | – | – | KR479665 | KR809685 | KR479841 |
| ZCMV5646 | Ankarafantsika | W1 | KR337955 | KR348302 | – | – | KR479666 | KR809686 | KR479842 |
| FGZC3011 | Ankarana | N | KR337877 | KR348306 | – | – | KR479683 | KR809622 | KR479770 |
| FGMV2002.840 | Ankarana | N | KR337868 | KR348303 | – | – | KR479637 | KR809613 | KR479760 |
| FGZC532 | Ankarana | N | AY848014 | KR348305 | KR337851 | KR337833 | KR479693 | KR809627 | KR479774 |
| FGZC533 | Ankarana | N | AY848015 | KR348304 | - | – | KR479694 | KR809628 | KR479775 |
| HER3497 | Ankatrakatraka Forest | W3 | KR337906 | KR479607 | – | – | – | KR809651 | KR479794 |
| HER3498 | Ankatrakatraka Forest | W3 | KR337907 | KR479608 | – | – | KR479738 | – | KR479795 |
| HER4021 | Ankavandra | W2 | KR337919 | KR479620 | – | – | KR479735 | KR809663 | KR479807 |
| HER4022 | Ankavandra | W2 | KR337920 | KR479621 | – | – | KR479732 | KR809664 | KR479808 |
| HER4023 | Ankavandra | W2 | KR337921 | KR479622 | – | – | KR479727 | – | KR479809 |
| HER4024 | Ankavandra | W2 | KR337922 | KR479623 | KR337849 | KR337837 | KR479724 | KR809665 | KR479810 |
| HER4048 | Ankavandra | W2 | KR337923 | KR479624 | – | – | KR479725 | KR809666 | KR479811 |
| HER4049 | Ankavandra | W2 | KR337924 | KR479625 | – | – | – | KR809667 | KR479812 |
| HER3713 | Antevankira Forest | W3 | KR337910 | KR479611 | – | – | KR479733 | KR809654 | KR479798 |
| HER3714 | Antevankira Forest | W3 | KR337911 | KR479612 | – | – | – | KR809655 | KR479799 |
| HER3715 | Antevankira Forest | W3 | KR337912 | KR479613 | – | – | KR479716 | KR809656 | KR479800 |
| FGZC1002 | Antsiranana | N | KR337869 | KR348307 | – | – | KR479680 | KR809614 | KR479762 |
| FGZC1319 | Antsiranana | N | KR337870 | KR348309 | – | – | KR479679 | KR809615 | KR479763 |
| FGZC1325 | Antsiranana | N | KR337871 | KR348308 | – | – | KR479636 | KR809616 | KR479764 |
| APR01013 | Bemaraha | W3 | KR337856 | KR479581 | – | – | KR479734 | KR809601 | KR479748 |
| APR01159 | Bemaraha | W2 | KR337857 | KR479582 | – | – | KR479721 | KR809602 | KR479749 |
| FGZC669 | Bemaraha | W2 | KR337888 | KR348310 | – | – | KR479684 | KR809633 | KR479780 |
| FGZC702 | Bemaraha | W2 | FJ559215 | KR479595 | – | – | KR479685 | KR809634 | KR479781 |
| FGZC732 | Bemaraha | W1 | KR337890 | KR348311 | – | – | KR479686 | KR809635 | KR479782 |
| FGZC733 | Bemaraha | W2 | KR337891 | KR348312 | – | – | KR479687 | KR809636 | KR479783 |
| FGZC749 | Bemaraha | W2 | KR337892 | KR348313 | – | – | KR479689 | KR809637 | KR479784 |
| FGZC903 | Bemaraha | W2 | KR337893 | KR348314 | KR337850 | KR337836 | KR479688 | KR809638 | KR479785 |
| FGZC936 | Bemaraha | W2 | KR337894 | KR348315 | – | – | KR479682 | KR809639 | KR479786 |
| HER2708 | Bemaraha | W2 | KR337899 | KR479600 | – | – | KR479737 | KR809644 | – |
| HER2750 | Bemaraha | W2 | KR337900 | KR479601 | – | – | KR479731 | KR809645 | KR479790 |
| HER2772 | Bemaraha | W2 | KR337901 | KR479602 | – | – | KR479739 | KR809646 | – |
| 2001E39 | Benavony | N | KR337854 | KR479579 | – | – | – | KR809599 | KR479745 |
| 2002B16 | Berenty | SC1 | KR337855 | KR479580 | – | – | KR479710 | KR809600 | KR479746 |
| HER3555 | Beronto Forest | W3 | KR337908 | KR479609 | – | – | KR479712 | KR809652 | KR479796 |
| HER3581 | Beronto Forest | W3 | KR337909 | KR479610 | – | – | – | KR809653 | KR479797 |
| FGZC59 | Ejeda | SC1 | KR337884 | KR348317 | – | – | KR479698 | KR809629 | KR479776 |
| FGZC60 | Ejeda | SC1 | AY848016 | KR348318 | – | – | KR479699 | KR809630 | KR479777 |
| FGZC61 | Ejeda | SC1 | KR337886 | KR348319 | – | – | KR479700 | KR809631 | KR479778 |
| FGZC62 | Ejeda | SC1 | KR337887 | KR348320 | – | – | KR479701 | KR809632 | KR479779 |
| FGZC50 | Ejeda | SC1 | KR337881 | KR348316 | – | – | KR479641 | KR809626 | KR479761 |
| FGMV2002.2075 | Ifaty | SW | KR337864 | KR479584 | KR337853 | KR337842 | KR479644 | KR809609 | KR479757 |
| FGMV2002.2076 | Ifaty | SC1 | KR337865 | KR479585 | – | – | KR479690 | KR809610 | KR479758 |
| FGMV2002.2077 | Ifaty | SC2 | KR337866 | KR479586 | KR337852 | KR337841 | KR479708 | KR809611 | KR479751 |
| FGZC32 | Ihosy | SC1 | AY848011 | KR479593 | – | – | KR479696 | KR809623 | KR479771 |
| FGZC33 | Ihosy | SC1 | AY848012 | KR479594 | – | – | KR479697 | KR809625 | KR479772 |
| HER4297 | Ingaro | W3 | KR337926 | KR479627 | – | – | KR479722 | KR809668 | KR479814 |
| HER4298 | Ingaro | W3 | KR337927 | KR479628 | – | – | KR479713 | KR809669 | KR479815 |
| HER4299 | Ingaro | W3 | KR337928 | KR479629 | – | – | KR479718 | KR809670 | KR479816 |
| HER4300 | Ingaro | W3 | KR337929 | KR479630 | – | – | KR479736 | – | KR479817 |
| HER4301 | Ingaro | W3 | KR337930 | KR479631 | – | – | KR479728 | – | KR479818 |
| HER4302 | Ingaro | W3 | KR337931 | KR479632 | – | – | KR479714 | – | KR479819 |
| HER4303 | Ingaro | W3 | KR337932 | KR479633 | – | – | KR479726 | – | KR479820 |
| FGZC338 | Isalo | SC1 | AY848013 | KR348327 | – | – | KR479702 | KR809624 | KR479773 |
| FGMV2002.1413 | Isalo | SC1 | KR337859 | KR348324 | – | – | KR479703 | KR809604 | KR479752 |
| FGMV2002.1419 | Isalo | SC1 | KR337860 | KR348325 | – | – | KR479704 | KR809605 | KR479753 |
| FGMV2002.1477 | Isalo | W3 | KR337861 | KR348326 | – | – | KR479705 | KR809606 | KR479754 |
| FGMV2002.1500 | Isalo | W3 | KR337862 | KR348328 | – | – | KR479706 | KR809607 | KR479755 |
| ZCMV5692 | Isalo | SC1 | KR337956 | KR348321 | – | – | KR479711 | KR809687 | KR479843 |
| ZCMV5693 | Isalo | SC1 | KR337957 | KR348322 | KR337845 | KR337840 | KR479667 | KR809688 | KR479844 |
| ZCMV5694 | Isalo | SC1 | KR337958 | KR348323 | – | – | KR479640 | KR809689 | KR479845 |
| ZCMV5695 | Isalo | SC1 | KR337959 | KR348331 | – | – | KR479668 | KR809690 | KR479846 |
| ZCMV5696 | Isalo | SC1 | KR337960 | KR348333 | – | – | KR479669 | KR809691 | KR479847 |
| ZCMV5712 | Isalo | SC1 | KR337961 | KR348334 | – | – | – | KR809692 | KR479848 |
| ZCMV5768 | Isalo | SC1 | KR337975 | KR348332 | – | – | KR479677 | KR809705 | KR479862 |
| ZCMV5770 | Isalo | SC1 | KR337976 | KR348330 | – | – | KR479678 | KR809706 | KR479863 |
| ZCMV5771 | Isalo | SC1 | KR337977 | KR348329 | KR337844 | KR337839 | KR479638 | KR809707 | KR479864 |
| HER3758 | Kapoky Forest | W3 | KR337913 | KR479614 | – | – | KR479730 | KR809657 | KR479801 |
| HER3792 | Kapoky Forest | W3 | KR337914 | KR479615 | – | – | KR479717 | KR809658 | KR479802 |
| HER3793 | Kapoky Forest | W3 | KR337915 | KR479616 | – | – | KR479729 | KR809659 | KR479803 |
| HER3814 | Kapoky Forest | SC2 | KR337916 | KR479617 | – | – | KR479740 | KR809660 | KR479804 |
| HER3815 | Kapoky Forest | W3 | KR337917 | KR479618 | – | – | KR479741 | KR809661 | KR479805 |
| HER3816 | Kapoky Forest | W3 | KR337918 | KR479619 | – | – | – | KR809662 | KR479806 |
| mir065 | Kirindy | W3 | KR337933 | KR348335 | – | – | KR479651 | KR809671 | KR479821 |
| mir066 | Kirindy | W3 | KR337934 | KR348336 | – | – | KR479652 | KR809672 | KR479822 |
| mir067 | Kirindy | W3 | KR337935 | KR348337 | – | – | KR479653 | KR809673 | KR479823 |
| mir085 | Kirindy | W3 | KR337936 | KR348338 | – | – | KR479649 | KR809674 | KR479824 |
| mir086 | Kirindy | W3 | KR337937 | KR348339 | – | – | KR479654 | KR809675 | KR479825 |
| mir119 | Kirindy | W2 | KR337938 | KR348340 | – | – | KR479659 | – | KR479826 |
| mir120 | Kirindy | W3 | KR337939 | KR348341 | KR337848 | KR337838 | KR479660 | KR809676 | KR479827 |
| mir121 | Kirindy | W3 | KR337940 | KR348342 | – | – | KR479661 | KR809677 | KR479828 |
| ZCMV12719 | Kirindy | W3 | KR337943 | KR348343 | – | – | KR479662 | KR809680 | KR479831 |
| ZCMV12756 | Kirindy | W3 | KR337944 | KR348344 | – | – | KR479647 | – | KR479832 |
| ZCMV12757 | Kirindy | W3 | KR337945 | KR348345 | – | – | KR479656 | KR809681 | – |
| ZCMV12758 | Kirindy | W3 | KR337946 | KR348346 | – | – | KR479639 | KR809682 | KR479833 |
| ZCMV12759 | Kirindy | W3 | KR337947 | KR348347 | – | – | KR479655 | KR809683 | KR479834 |
| ZCMV12760 | Kirindy | W3 | KR337948 | KR348348 | – | – | KR479657 | – | KR479835 |
| ZCMV12761 | Kirindy | W3 | KR337949 | KR348349 | – | – | KR479658 | – | KR479836 |
| ZCMV12779 | Kirindy | W3 | KR337950 | KR348350 | – | – | KR479648 | – | KR479837 |
| ZCMV12780 | Kirindy | W3 | KR337951 | KR348351 | – | – | KR479646 | – | KR479838 |
| FRC570 | Marosely | W1 | KR337895 | KR479596 | – | – | – | KR809640 | KR479787 |
| FRC571 | Marosely | W1 | KR337896 | KR479597 | – | – | – | KR809641 | KR479788 |
| RF442 | Marosely | W1 | KR337942 | KR479634 | KR337846 | KR337835 | – | KR809679 | KR479830 |
| HER2871 | Soatanana | W1 | KR337903 | KR479604 | – | – | KR479742 | KR809648 | – |
| FGMV2002.1535 | Toliara | W3 | KR337863 | KR348357 | – | – | KR479707 | KR809608 | KR479756 |
| ZCMV5716 | Toliara | W3 | KR337962 | KR348352 | – | – | – | KR809693 | KR479849 |
| ZCMV5717 | Toliara | SW | KR337963 | KR348353 | – | – | KR479650 | KR809694 | KR479850 |
| ZCMV5718 | Toliara | SW | KR337964 | KR348354 | – | – | KR479681 | KR809695 | KR479851 |
| ZCMV5735 | Toliara | SC1 | KR337965 | KR348355 | – | – | KR479670 | – | KR479852 |
| ZCMV5736 | Toliara | SC1 | KR337966 | KR348356 | – | – | KR479671 | KR809696 | KR479853 |
| ZCMV5737 | Toliara | SW | KR337967 | KR348358 | – | – | KR479672 | KR809697 | KR479854 |
| ZCMV5738 | Toliara | SC1 | KR337968 | KR348359 | – | – | KR479673 | KR809698 | KR479855 |
| ZCMV5739 | Toliara | W3 | KR337969 | KR348360 | – | – | – | KR809699 | KR479856 |
| ZCMV5740 | Toliara | SC1 | KR337970 | KR348361 | – | – | – | KR809700 | KR479857 |
| ZCMV5741 | Toliara | SC1 | KR337971 | KR348362 | – | – | KR479674 | KR809701 | KR479858 |
| ZCMV5742 | Toliara | W3 | KR337972 | KR348363 | – | – | KR479675 | KR809702 | KR479859 |
| ZCMV5743 | Toliara | SC2 | KR337973 | KR348364 | – | – | KR479676 | KR809703 | KR479860 |
| ZCMV5744 | Toliara | W3 | KR337974 | KR348365 | – | – | KR479643 | KR809704 | KR479861 |
| FGZC2331 | Tranomaro | SC1 | KR337874 | KR479590 | – | – | KR479691 | KR809619 | KR479767 |
| FGZC2332 | Tranomaro | SC1 | KR337875 | KR479591 | – | – | KR479692 | KR809620 | KR479768 |
| DRV6059 | Tsaratanana | W1 | KR337858 | KR479583 | – | – | – | KR809603 | KR479750 |
| HER4264 | Vohitsira | W3 | KR337925 | KR479626 | – | – | KR479720 | – | KR479813 |
| ZCMV770 | *Aglyptodactylus* | na | KR337978 | AB325874\* | KR337843 | KR337832 | KR479635 | – | KR479747 |

\*used sequence from different specimen in GenBank

**Table S2**. Primer sequences and PCR conditions. PCR conditions start with temperature (°C) of each step followed by the time in brackets (seconds). Reactions were performed in a final volume of 11 µl, using 0.3 µl each of 10 pmol primers, 0.25 µl of total dNTP 10 mM (Promega), 0.08 µl of 5 U/ml GoTaq, and 2.5 µl 5X GoTaq Reaction Buffer (Promega). Purification of the PCR products was performed by Exonuclease I and Shrimp Alkaline Phosphatase digestion. The amplicons were sequenced using BigDye v3.1 cycle sequencing chemistry with forward PCR primers for mtDNA genes, and both forward and reverse primers in the case of nuclear genes. Sequencing products were run on a 3130xl genetic analyzer (Applied Biosystems). Sequences were edited and aligned in CodonCode Aligner v3.0.3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Primer name** | **Sequence (5'-3')** | **Source** | **PCR conditions** |
| **12S rRNA** | 12SAL (F) | AAACTGGGATTAGATACCCCACTAT | Kocher et al. (1989), modified | 94(120), [94(20), 50(45), 72(120) x 34] 72(600) |
| **12S rRNA** | 12SBH (R) | GAGGGTGACGGGCGGTGTGT | Kocher et al. (1989), modified |
| **16S rRNA** | AC16SAR (F) | CGCCTGTTTATCAAAAACAT | Palumbi et al. (1991), modified | 94(120), [94(20), 53(45), 72(120) x 40] 72(600) |
| **16S rRNA** | AC16SBR (R) | CCGGTYTGAACTCAGATCAYGT | Palumbi et al. (1991), modified |
| ***cytb*** | CytB-A MP4 (F) | TACCCTGAGGWCAAATATCTTTC | Bossuyt and Milinkovitch (2000), modified | 94(120), [94(20), 55(45), 72(120) x 36] 72(600) |
| ***cytb*** | CytB-C MP1 (R) | GAAATAGAGCCCGGAGGCTAC | Bossuyt and Milinkovitch (2000), modified |
| ***cox1*** | LCO 1490 (F) | GGTCAACAAATCATAAAGATATTGG | Folmer et al. (1994) | 94(120), [94(20), 50(45), 72(120) x 34] 72(600) |
| ***cox1*** | HCO 2198 (R) | TAAACTTCAGGGTGACCAAAAAATCA | Folmer et al. (1994) |
| ***bdnf*** | BDNF\_F2 (F) | CCATCCTTTTCCTKACTATGGT | Vieites et al. (2007), modified | 94(120), [94(20), 52(45), 72(120) x 40], 72(600) |
| ***bdnf*** | BDNF\_R2 (R) | TATCTTCCCCTTTTAATGGTCA | Vieites et al. (2007) , modified |
| ***pomc*** | POMC\_DRV\_F1 (F) | ATATGTCATGASCCAYTTYCGCTGGAA | Vieites et al. (2007) | 94(120), [94(20), 55(45), 72(120) x 36], 72(600) |
| ***pomc*** | POMC\_DRV\_R1 (R) | GGCRTTYTTGAAWAGAGTCATTAGWGG | Vieites et al. (2007) |
| ***rag1*** | RAG1 FIII (F) | TGGCACAGGGTATGATGARA | Pabijan et al. (2011) | 94(120), [94(20), 54(45), 72(180) x 38], 72(600) |
| ***rag1*** | RAG1 RIII (R) | TCAATGATCTCTGGGACGTG | Pabijan et al. (2011) |

**Table S3**. Uncorrected *p*-distances between major mtDNA phylogroups in *L. labrosum* in the 16S rRNA gene (550-555 bp; below diagonal) and *cytb* gene (625 bp; above diagonal). N-northern mtDNA clade; W1 –west 1; W2 – west 2; W3 – west 3; SC1 – southcentral 1; SC2- southcentral 2; SW- southwestern; see Fig. 2 and Fig. 3 for mtDNA clade definitions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **N** | **W1** | **W2** | **W3** | **SC1** | **SC2** | **SW** |
| **N** |  | 0.028 | 0.025 | 0.028 | 0.051 | 0.054 | 0.065 |
| **W1** | 0.004 |  | 0.011 | 0.014 | 0.049 | 0.052 | 0.061 |
| **W2** | 0.005 | 0.004 |  | 0.010 | 0.045 | 0.048 | 0.058 |
| **W3** | 0.004 | 0.003 | 0.002 |  | 0.046 | 0.052 | 0.057 |
| **SC1** | 0.009 | 0.007 | 0.004 | 0.006 |  | 0.010 | 0.047 |
| **SC2** | 0.005 | 0.004 | 0.004 | 0.002 | 0.007 |  | 0.050 |
| **SW** | 0.009 | 0.007 | 0.008 | 0.006 | 0.007 | 0.007 |  |

**Table S4**. Results of the spatial analysis of molecular variation (SAMOVA) for *K*=2-6. Percentage of genetic variation explained by each covariance component: among group variation (Va), variation among populations within groups (Vb), and variation within populations (Vc). All values are significant at *P*<0.00001. Note that the change in Va stabilizes at *K*=3.

|  |  |  |  |
| --- | --- | --- | --- |
| ***K*** | **Va** | **Vb** | **Vc** |
| 2 | 57.1 | 13.7 | 29.2 |
| **3** | **61.9** | **7.8** | **30.3** |
| 4 | 63.0 | 5.3 | 31.7 |
| 5 | 63.7 | 2.8 | 33.5 |
| 6 | 64.3 | -1.64 | 37.3 |

**Table S5**. Percentage of variation in the nuclear genes explained by among group variation (Va), variation among populations within groups (Vb), and variation within populations (Vc). The groups in this hierarchical AMOVA were defined according to the SAMOVA results for mtDNA. All values are significant at *P*<0.001 except Va for *pomc* (marked with a \* symbol).

|  |  |  |  |
| --- | --- | --- | --- |
| **gene** | **Va** | **Vb** | **Vc** |
| ***bdnf*** | 45.5 | 15.2 | 39.3 |
| ***pomc*** | -1.15\* | 11.0 | 90.2 |
| ***rag1*** | 10.0 | 11.2 | 78.7 |

**Table S6**. Overall *F*ST values across all populations (*F*ST pop) and among major mtDNA clades (*F*ST clades) calculated for mtDNA and 3 nuclear genes. All values were significant at *P*<0.0001 except for *pomc* differentiation among mtDNA clades (*P*=0.0694, marked with a \* symbol).

|  |  |  |
| --- | --- | --- |
| **gene** | ***F*ST pop** | ***F*ST clades** |
| **mtDNA** | 0.585 | 0.871 |
| ***bdnf*** | 0.512 | 0.425 |
| ***pomc*** | 0.102 | 0.014\* |
| ***rag1*** | 0.177 | 0.121 |

**Table S7**. Pairwise *F*ST values for 3 nuclear genes (*bdnf*/*pomc*/*rag1*) among *Laliostoma labrosum* individuals grouped according to major mtDNA clade. Red highlighting indicates significant differentiation at *P*<0.05. N-northern mtDNA clade; W1 –west 1; W2 – west 2; W3 – west 3; SC1 – southcentral 1; SC2- southcentral 2; SW- southwestern; see Fig. 2 and Fig. 3 for mtDNA clade definitions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **N** | **W1** | **W2** | **W3** | **SC1** | **SC2** |
| **W1** | 0.75/0/0.24 |  |  |  |  |  |
| **W2** | 0.75/-0.01/0.35 | 0.08/0/0.09 |  |  |  |  |
| **W3** | 0.77/0.01/0.378 | 0.14/0.01/0.08 | -0.01/0.01/0.03 |  |  |  |
| **SC1** | 0.82/0.02/0.52 | 0.47/0.02/0.15 | 0.21/0.02/0.05 | 0.14/0.02/0.02 |  |  |
| **SC2** | 0.71/0.34/0.5 | -0.02/0.09/0 | -0.01/0.12/-0.09 | 0.03/0/-0.05 | 0.37/0.02/-0.01 |  |
| **SW** | 0.74/0.09/0.62 | 0/0/0.217 | -0.06/-0.05/-0.05 | -0.03/-0.03/0.06 | 0.28/-0.03/0.1 | -0.09/-0.01/-0.09 |

**Fig. S1.** Majority rule consensus tree based on a posterior of 25000 trees from a mixed-model Bayesian analysis of the two-gene dataset (concatenated 16S rRNA and *cytb* genes) from all *L. labrosum* samples. Posterior probabilities over 0.70 are given above branches:\* denotes values ≥ 0.9 and < 0.95; \*\* denotes posterior probabilities ≥0.95.



**Fig S2**. Summary of Structure simulations for *K* values from 1 to 10 (each simulation repeated 20 times) based on variation in 3 nuclear genes and mtDNA in *L. labrosum*. Estimated Ln probability of data for a given *K* and the Δ*K* value (Evanno et al., 2005) from Structure Harvester (Earl and vonHoldt, 2012).



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