SUPPORTING INFORMATION

Genome-wide nuclear data confirm two species in the Alpine endemic land snail *Noricella oreinos* s.l. (Gastropoda, Hygromiidae)

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Method S1

Sequence generation – Control reactions for DNA extraction and PCR amplification were included in mt COI amplification reactions to detect contaminations. The COI amplification reactions were performed following the QIAGEN TopTaq manufacturer’s protocol with 1x TopTaq PCR Buffer, 1x Q-Solution™, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.5 U TopTaq DNA polymerase, 1 µl template DNA and nuclease free water to a total volume of 25 µl. PCR conditions were: 94°C for 3 min, 35 cycles of 94°C for 30 sec, 54°C for 30 sec, 72°C for 30 sec, followed by 72°C for 7 min.

The ITS2 sequences were amplified using the TopTaq DNA polymerase (Qiagen). In case of length polymorphisms or more than one allele at the ITS2 locus, direct sequencing of the PCR was unsuccessful. PCR reactions were repeated from such specimens using the Q5® High-Fidelity DNA polymerase (NEB). The ITS2 master mix included 1x Q5 Reaction Buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.5 U Q5 High-Fidelity DNA Polymerase, 0.5 µl template DNA and nuclease free water to a total volume of 25 µl. PCR conditions were: 98°C for 30 sec, 30 cycles of 98°C for 10 sec, 63°C for 20 sec, 72°C for 30 sec, followed by 72°C for 7 min. Subsequent cloning and sequencing was performed according to Kruckenhauser et al. (2014). In general, up to four clones per individual were sequenced. From the nine individuals which possessed the ITS2 allele of both N. oreinos subspecies, DNA extraction and cloning of the ITS2 fragment was repeated. Cloned sequences that occurred more than once for an individual were removed in the final dataset.

Method S2

Increasing DNA concentration for AFLP generation – If the concentration of available or newly-extracted DNA samples was below 10 ng/µl, the samples were post-processed: The AE-based DNA samples were precipitated using a conventional protocol, whereas samples, which had been eluted in nuclease free water, were concentrated using a vacuum centrifuge (UniEquip UniVapo 150 ECH). For precipitation, 1/10 Vol. 3M sodium acetate (pH 5.2), 9/10 Vol. DNA and 3x Vol. 96% ethanol were used according to a standard precipitation protocol. The pellet was dissolved in 15-30 µl nuclease free water (Qiagen). For the vacuum centrifuge concentration, DNA samples were incubated at 50°C for 3.25 hours and 15 µl sterile ddH₂O were added.

Method S3

Amplified Fragment Length Polymorphism – The various steps of restriction-ligation, PCR amplification and purification were carried out simultaneously for 48 samples in a GeneAmp® PCR System 9700 thermocycler (Thermo Fisher Scientific Inc.). Unless otherwise indicated, all chemicals
were purchased from VWR. The initial selective PCR primer pairs were chosen after a primer test using samples of 16 specimens and nine selective primer pair combinations with three selective nucleotides each (data not shown).

For each reaction, 5 µl of template DNA (minimum of 50 ng DNA) was digested for three hours at 37 °C with 6 µl of the corresponding master mix. The master mix contained 1x T4 Ligase Buffer (Promega), 50 mM NaCl, 0.55 µg BSA (Promega), 4.55 µM Msel adaptor (MWG Eurofins, Germany), 0.46 µM EcoRI adaptor (MWG Eurofins), 1 U Msel enzyme (Promega), 8 U EcoRI enzyme (Promega), 0.9 U T4 DNA Ligase (Promega) and sterile ddH2O. Ten percent of the DNA samples were selected randomly and repeated from the restriction-ligation step onwards to test the repeatability of the subsequently scored AFLP fragments and to estimate the error rate. Additionally, each of the six restriction ligation plates contained a control reaction with no template and one sample chosen for repetition.

The pre-selective amplification reactions were performed using PCR primer pairs with one selective base at the 3'-end of each primer. The 10 µl reaction consisted of 2 µl of the 20-fold diluted restriction-ligation template, 1x Green GoTaq buffer (Promega), 0.22 mM dNTPs, 0.29 µM EcoRI-A primer (MWG Eurofins), 0.29 µM Msel-C primer (MWG Eurofins) and 0.125 U GoTaq G2 (Promega). Pre-selective PCR conditions were as follows: 72°C for 2 min, 30 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min, finishing with 72°C for 10 min.

For amplification in selective PCR reactions, primer pairs with three selective bases at the 3'-end of each PCR primer were used. The following combinations of fluorophore-labelled EcoRI primers and unlabelled Msel primers were used: EcoRI-ATG<sup>6</sup>FAM/Msel-CTT, EcoRI-AGG<sup>VIC</sup>/Msel-CTC and EcoRI-ACA<sup>NED</sup>/Msel-CAA. The selective amplification was carried out within 10 µl of 1x Green GoTaq buffer, 0.22 mM dNTPs, each 0.27 µM of the respective EcoRI-NNN primer (Applied Biosystems) and Msel-NNN primer (MWG Eurofins), 0.2 U GoTaq G2, sterile ddH2O and 2 µl of the 20-fold diluted pre-selective PCR product. For all three primer pairs, selective amplification was performed with the following cycling conditions: 94°C for 2 min, 10 cycles of 94°C for 20 sec, 66°C (decreasing 1°C in each cycle) for 30 sec, and 72°C for 2 min, followed by 20 cycles of 94°C for 20 sec, 56°C for 30 sec, and 72°C for 2 min, and a final step of 60°C for 30 min.

Following the protocol of (Bendiksby, Tribsch, Borgen, Trávníček, & Brysting, 2011), 6 µl of the NED-labelled PCR products were pooled with both 3 µl of FAM and VIC-labelled product and purified using Sephadex<sup>™</sup> G-50 Superfine resins (GE Healthcare BioSciences). For MegaBACE preparation, 12.9 µl AD, 0.1 µl ET-ROX 400-R MegaBACE size standard and 2 µl (in case of the first run) or respective 1.6 µl of the purified PCR products were denatured at 95°C for 3 min and immediately cooled on ice until capillary electrophoresis.
Table S1. Primer combinations for COI and ITS2 amplification.

| Primer 5'-3'       | Origin                                                | Fragment size (bp) |
|--------------------|-------------------------------------------------------|--------------------|
| **COI** folmerfwd: | Duda et al. (2011) modified from Folmer et al. (1994) | 705                |
| GGTCAACAATCATAAAGATATTGG |                                                        |                    |
| **COI** schneckrev:| Duda et al. (2011), modified from Gittenberger et al. (2004) |          |
| TATACCTCTGGATGACCAAAAAATCA |                                                        |                    |
| **LSU-1:**         | Wade & Mordan (2000)                                  |                    |
| CTAGCTGCGAGAATTAATGTGA |                                                        |                    |
| **LSU-3:**         | Wade & Mordan (2000)                                  | up to 954          |
| ACTTTCCCTCACGTTCTTG |                                                        |                    |

Table S2. List of the AFLP datasets. The number of mountain regions (Nreg), sampling localities (Nloc), specimens (N) and AFLP markers (N_AFLP) are included with the applied programs or scripts (Splitstree, Past3, GenAlEx, AFLPdat.R, STRUCTURE). The number of K chosen for STRUCTURE computations is given. The datasets D* and E1* were generated for AMOVA calculations by excluding localities with single samples from the datasets D and E. For hierarchical STRUCTURE cluster analyses, three subsets E2-E4 were generated as specified in the text.

| Dataset          | Nreg | Nloc | N | N_AFLP | Splitstree | PAST3 | GenAlEx | AFLPdat.R | STRUCTURE |
|------------------|------|------|---|--------|------------|-------|---------|-----------|-----------|
| N. oreinos, T. hispidus (outgroup) |      |      |   |        |            |       |         |           |           |
| dataset A        | 36   | 208  | 329|        | X          |       |         |           |           |
| N. oreinos – complete dataset (mountain regions M2-M15) |      |      |   |        |            |       |         |           |           |
| dataset B        | 14   | 32   | 202| 316    | X          | X     |         |           | 1-20      |
| dataset B*       | 14   | 29   | 199| 316    | X          | X     |         |           | 1-20      |
| Supposed contact zone (M5, M6) of N. oreinos subspecies and adjacent mountain (M8) |      |      |   |        |            |       |         |           | 1-7       |
| dataset C        | 3    | 7    | 114| 292    |            |       |         |           | 1-7       |
| N. o. scheerpeltzi (M2-M5) |      |      |   |        |            |       |         |           | 1-10      |
| dataset D        | 4    | 15   | 50 | 263    | X          |       |         |           | 1-10      |
| dataset D*       | 4    | 13   | 48 | 261    | X          | X     |         |           | 1-10      |
| N. o. oreinos (M6-M15) |      |      |   |        |            |       |         |           | 1-15      |
| dataset E1       | 10   | 17   | 152| 293    | X          |       |         |           | 1-15      |
| dataset E1*      | 10   | 16   | 151| 293    | X          | X     |         |           | 1-15      |
| N. o. oreinos – dataset E1 without Haller Mauern (M7-M15) |      |      |   |        |            |       |         |           | 1-15      |
| dataset E2       | 9    | 13   | 72 | 274    |            |       |         |           | 1-15      |
| N. o. oreinos – Gesäuse mountain regions (M7-M10) |      |      |   |        |            |       |         |           | 1-7       |
| dataset E3       | 4    | 5    | 43 | 247    |            |       |         |           | 1-7       |
| N. o. oreinos – Hochschwab to Schneeberg (M11-M15) |      |      |   |        |            |       |         |           | 1-7       |
| dataset E4       | 5    | 8    | 29 | 238    |            |       |         |           | 1-7       |
Table S3. Genetic diversity calculated for the complete AFLP dataset A and the two *Noricella* taxa (datasets D* and E1*). For each locality, the number of specimens N, the mean observed number of AFLP fragments \( N_A \), the percentage of polymorphic fragments PPF and the Nei’s gene diversity (D) are given.

| Locality  | N   | \( N_A \) | PPF   | D   |
|-----------|-----|----------|-------|-----|
| N. oreinos| 199 | 1.94     | 93.7  |     |
| N. o. scheerpetzi | 48  | 1.81     | 81.2  |     |
| 382       | 2   | 0.66     | 6.1   | 0.061|
| 383       | 2   | 0.77     | 13.4  | 0.134|
| 389       | 2   | 0.65     | 5.0   | 0.050|
| 387       | 2   | 0.61     | 2.3   | 0.023|
| 765       | 5   | 0.86     | 17.2  | 0.081|
| 766       | 2   | 0.77     | 10.0  | 0.100|
| 767       | 2   | 0.70     | 8.8   | 0.088|
| 768       | 2   | 0.81     | 12.3  | 0.123|
| 769       | 2   | 0.79     | 14.9  | 0.149|
| 369       | 4   | 0.87     | 18.8  | 0.104|
| 367       | 4   | 0.80     | 14.9  | 0.082|
| 443       | 12  | 1.18     | 40.6  | 0.128|
| 444       | 7   | 1.12     | 36.8  | 0.137|

Localities mean 4 0.81 15.5 SE 0.0 0.01 3.2

| Locality  | N   | \( N_A \) | PPF   | D   |
|-----------|-----|----------|-------|-----|
| N. o. oreinos | 151 | 1.83     | 82.6  |     |
| 783       | 18  | 1.01     | 28.3  | 0.076|
| 782       | 23  | 1.13     | 35.8  | 0.083|
| 784       | 11  | 1.11     | 33.1  | 0.101|
| 785       | 28  | 1.06     | 32.8  | 0.076|
| 55        | 5   | 0.85     | 17.1  | 0.072|
| 665       | 6   | 0.92     | 22.2  | 0.071|
| 781       | 15  | 1.06     | 32.1  | 0.090|
| 779       | 10  | 0.87     | 21.8  | 0.076|
| 399       | 7   | 0.89     | 21.8  | 0.085|
| 134       | 7   | 0.98     | 29.7  | 0.126|
| 588       | 3   | 0.71     | 13.0  | 0.086|
| 338       | 8   | 0.81     | 19.8  | 0.074|
| 737       | 2   | 0.68     | 7.2   | 0.072|
| 79        | 4   | 0.67     | 8.5   | 0.046|
| 448       | 2   | 0.60     | 4.1   | 0.041|
| 178       | 2   | 0.63     | 5.8   | 0.058|

Localities mean 9 0.87 20.8 SE 0.1 0.01 2.7
Fig. S1. Neighbor-joining tree inferred from mitochondrial COI sequences. Colours indicate *N. o. oreinos* (pink) and *N. o. scheerpeltzi* (blue). Bootstrap values >75% are given at the nodes. The scale bar represents 0.02 substitutions expected per aligned positions. One sequence of *T. hispidus* (black) was used as outgroup.
Fig. S2. Scatter plots of the first and second axis of the PCoA based on Jaccard similarity estimates calculated for the dataset A. In total, 152 specimens of *N. o. oreinos* (pink dots), 50 specimens of *N. o. scheerpeltzi* (blue dots) and six specimens of *T. hispidus* used as outgroup (black triangles) were analysed. The axes are scaled by the square root of the Eigenvalues. Eigenvalues and variance represented by the first two axes are indicated at the axes (a) and Eigenvalues of the first ten axes are illustrated (b). Specimens of the potential contact zone (M6 region, Haller Mauern east) are drawn with dark pink dots. The specimens found heterozygous, possessing the *ITS2* alleles of both taxa (violet dots), are clustering within the M6 region (black arrow).
Fig. S3. Mean and SD values of the ln Pr(X|K) estimates and the ΔK values for each K as computed in STRUCTURE for the datasets B-D.
Fig. S4. Mean and SD values of the ln Pr(X|K) estimates and the ΔK values for each K as computed in STRUCTURE for the datasets E1-E4.
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