

Supplementary files

Title of the manuscript:

From hybrid to hemiclone: The genetic basis and evolutionary significance of clonal reproduction in *Pelophylax esculentus*

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Supplementary Table S1: Overview of adult *Pelophylax* water frog taxa used in this study.

Taxon name	Country of origin	Number of individuals	Sex
<i>P. ridibundus</i>	Czech Republic	10	5 males, 5 females
<i>P. ridibundus</i>	Slovakia	1	female
<i>P. lessonae</i>	Czech Republic	10	5 males, 5 females
<i>P. lessonae</i>	Germany	1	female
<i>P. esculentus</i> (RL)	Czech Republic	10	5 males, 5 females
<i>P. esculentus</i> (RLL)	Ukraine	2	?
<i>P. esculentus</i> (RRL)	Ukraine	2	?
<i>P. kurtmuelleri</i>	Albania	8	4 males, 4 females
<i>P. shqipericus</i>	Albania	6	3 males, 3 females
<i>P. epeiroticus</i>	Greece	6	3 males, 3 females
<i>P. bergeri</i>	Italy	6	3 males, 3 females
<i>P. perezi</i>	France	6	3 males, 3 females
<i>P. perezi</i>	Spain	5	3 males, 2 females

Notes: RL, diploid hybrid *P. esculentus*; RLL, triploid *P. esculentus* with one haploid set from *P. ridibundus* and two haploid sets from *P. lessonae*; RRL, triploid *P. esculentus* with two haploid sets from *P. ridibundus* and one haploid set from *P. lessonae*.

Supplementary Table S2: Overview of *Pelophylax* water frog families from artificial crossing experiments.

			Mother's		Father's	
Family ID	Genotype (all diploids; numbers of tadpoles are given in parenteses)	Sex	Genotype	System, country of origin, city/village	Genotype	System, country of origin
21-2020	<i>P. esculentus</i> (2)	?	<i>P. ridibundus</i>	R-R, Czech Republic, Cítov	<i>P. lessonae</i>	L-E, Czech Republic, Trnávka
37-2020	<i>P. esculentus</i> (2)	?	<i>P. ridibundus</i>	R-R, Czech Republic, Plzeň	<i>P. lessonae</i>	L-E, Czech Republic, Starý Smolivec
12-2022	<i>P. esculentus</i> (9)	?	<i>P. ridibundus</i>	R-R, Czech Republic, Cítov	<i>P. lessonae</i>	L-E, Slovakia, Zubrohlava
27-2021	<i>P. esculentus</i> (2)	?	<i>P. lessonae</i>	L-E, Czech Republic, Dlouhá, Karlovy Vary region	<i>P. ridibundus</i>	R-E, Czech Republic, Jistebník
57-2020	<i>P. esculentus</i> (3)	?	<i>P. esculentus</i>	L-E, Czech Republic, Trnávka	LL	L-E, Czech Republic, Starý Smolivec

Supplementary File S1

a) Sequence reads of the three designed markers specified in Table 2.

>Pelophylax-lessonae_Pll_Cl-289_minisat_consensus_REx2

TGACCAAAAACGACACTCCCGGCCCTTGGAAGCCAAAAATGGTAATTT

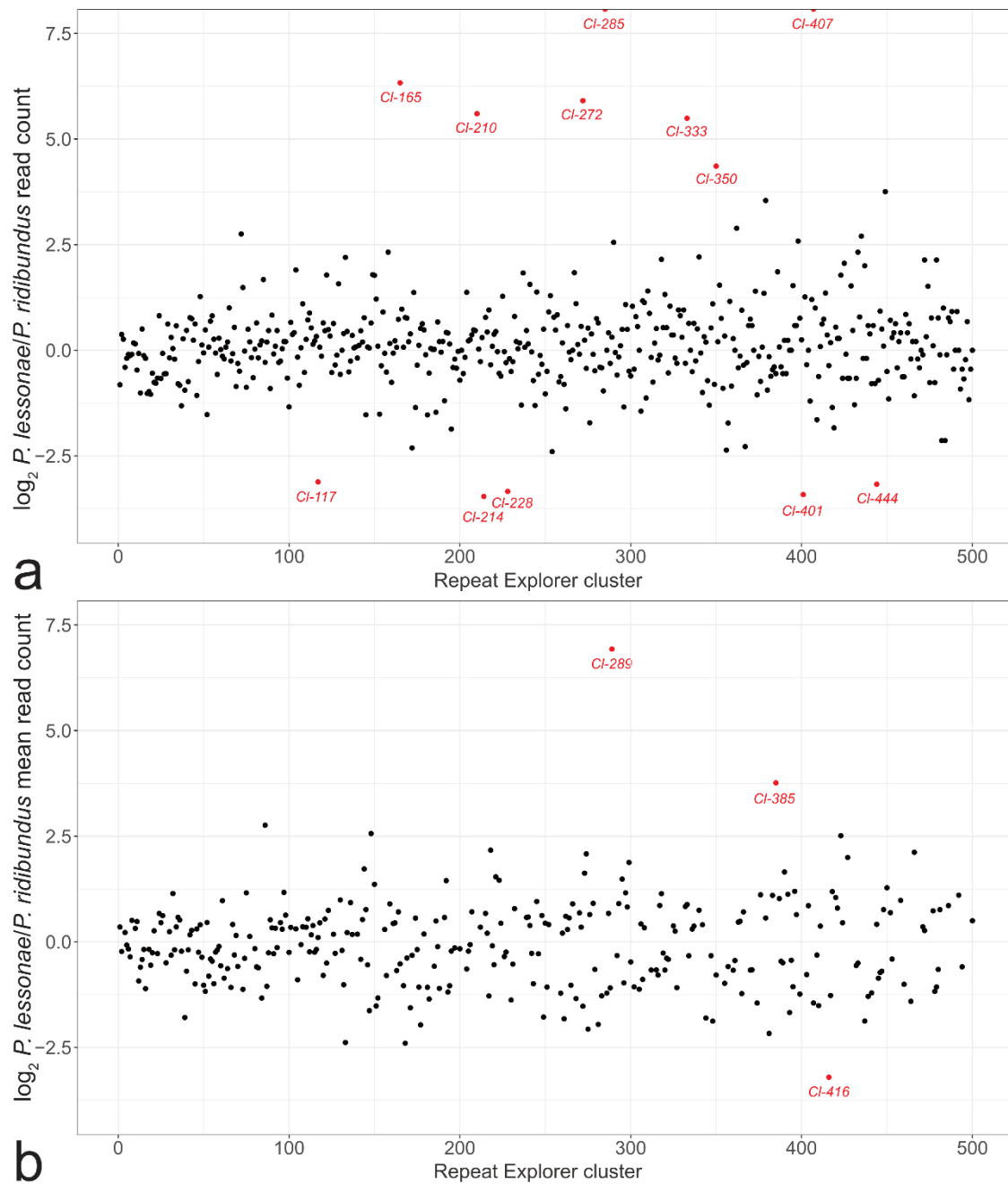
>Pelophylax-lessonae_Pll_Cl-385_sat_consensus_REx2

GCGAGCGAAAGCTACGCCTTGGGGGGCACCCTTTTGTGGCCCAGGCCCCGAATGG
ACACACTTCTCACGGGCGGTAAGCACACCATCTCATCTCATCCGTGTTTGGTCGG
GGTGTGTGTATTTATATATAAATGTAATTTTTTTTTTCCCCTTTTCTTTTCTGTTTTT
GCCACAGTGGAAGTTTATTATGGGGCGGCCC

>Pelophylax-ridibundus_Prr_Cl-416_LTR_longestTig_REx2

TGTAACAGATGTGTTCTGTAAACATTTACCTTTGCTGGTCTGTGCAGTCCGTCTAG
ACATACCTCTCCAACGATGACCCATCCGAGATCAAGGCGTTGTGCAAATGGTGCG
TTGTGTGGCCCATTTGTAAGTGTCTCTGACTTTGTGCACTCTGAGGATATCTCTCCC
AAGTAGAAGGGTGATTGCAGCATCTGGATCTAGTGCTGGTATCTGGTCGGCTATT
CTCACTAGATGAGGGTGATGACGAGCAACTTCAGGTGTGGGGATCTCTGACTTGT
CGTCTGGTATCTCATCACATTCTATGAGGGTAGGAAGAGTCACCTTTGTCTTTCCA
TCTAATGACTCGATGATGTAGTTGTTTGCTCTTCTCCCTGTCGTCTCTACAACTCC
AGAACAAGTCTTCAAGGTGTATGGAGTTGGACTTCCTTTGTCACCGAAGAGGTCA
AAGAACTCTGTTTTTGCCAGAGATCTGTTACTCTGTTTCATCCAAGACTGTGTACAT
CTTGATTGCCTTTTCTCTGAAACCTGCAGGATACACCTTGACTAAGCATATCTTGG
AGCATGATCTAGGACGGCCCTGCTCTGTACAGATCTCTGTGCACTTGGAGGATAC
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TTGCACTCTGTGCATTGTATGGTTTTTGTACAGTCTTTTGCAAGATGTTGAATTGA
AGCACAACATCTGAAACAAATGCGATTGTCTTTAAGGTAAGCTTTGCGCTCTTCT
AATGTCTTACTTCTGAAACTGCGACATTTTCTTAGTGGGTGAGGCTTGTTGTGGAT
CGGACATATTTTGTCTGGCTCCTCCACTTTCTTACTTGCGTAGTCTTCCTGATTGGC
TGCAGATTCAGTAGGGACTTCTGTCTTCCGTACAGAACTGTTGCTCTGCGTTCTT
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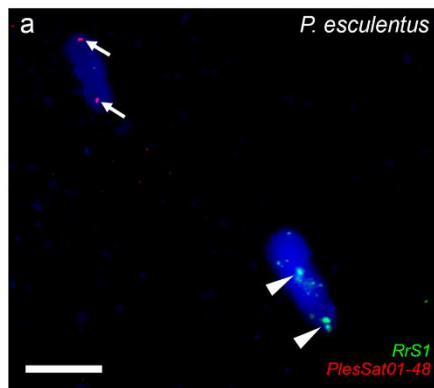
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TTGAGTGACCCACCTTTCTTGCAAGTTGTAAGGAAGTTTCTCGATTATGGGATCAA
TTCCACGTGCTGTATCCAAGTATGAGAGACCTGGCAGATATCCACTAGACTTAGC
GGCTTCTATTTCTAAGAGTAGGTCCCCCAAACCTCTCAGCTTCTGATTGTCCTTGT
TTGTTATTCTTGGATAGTTTTCTATCCTCTTCAGAAGCGCATCCTCGATCACTTCA
GGTGATCCATAGCATTGTTCTAGTCTCTGCCACACCATWGCAAGTCCTGCTGCTG
CATCATGAACATGTACTGACCTGATTCTTTGGGCTTGCTCAGTGGAYTCTGGTCCT
AGCCATTTGACGAGCTGGTCAAGCATTCTCTGTCTGTCAGATCTAAGTCTTGGGT
ACCGCTCAGGAAAGAGGACTTCCAAGGCCAGTAGTTTTCTGGACGGTCATCGAAT
TTCATGAGACCAGCGCTCACGATCTCACGGCGCATCAGGAATCTTGCAACTTCTG
TAGCTCTTGCTGCGTCTGGTGAATACTTCCTGGGTGTAAACTCAGGGTATGGCTGC
GGCTGGTAAGGCTTATTAGATGCTTGACTAAGTCCATTTTGGTTAGGTTGCTGTTC
TCGTCTGTTGTAAATCTTTCTGCTATAATCCAGGCTTGTATTCGCTTGAGGAGGGA
TTACATCAGCATCTGCTTGCACTCTTGGAGGGTTGGCAGATTGGTCTCTGAGCATG
GACCACTTGACTCCTCATGCTGAGGTTCTGATTCAGTCTTGCRGKGTGGMGTMRN
TGACGACCTAGGTATGTCATTGGAC



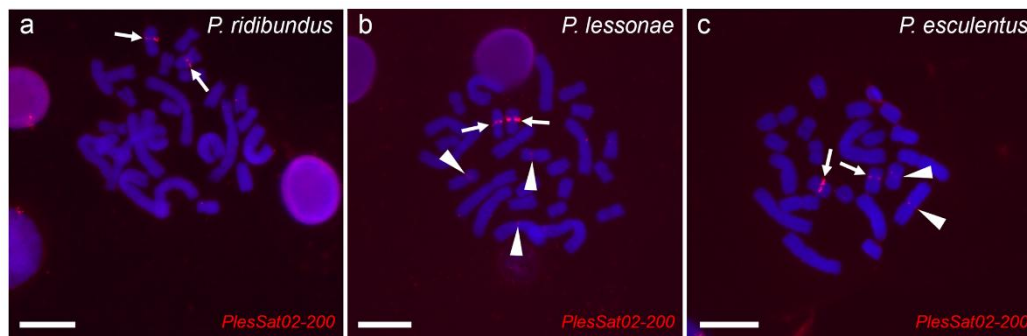
Supplementary Figure S1. RepeatExplorer comparative analyses of repeats between *Pelophylax lessonae* and *P. ridibundus*. (a) The first run identified seven highly enriched repeat clusters in *P. lessonae* and five in *P. ridibundus* (in red). (b) Plot shows only significantly enriched repeat clusters identified in the second run with three independent read samples from the sequencing data after excluding the 25 most abundant clusters. Highly enriched clusters CI-289, CI-385, and CI-416 (in red). Note that CI-165 and CI-210 from the first run correspond to CI-289 and CI-385, respectively, from the second run.

Forthcoming on Dryad. <https://doi.org/10.5061/dryad.v41ns1s5s>

<http://datadryad.org/stash/share/SNauTIZ7UNRJQMcyjxy1PKGjLt9NsxGHnnes00tPHf4>



Supplementary Figure S2. Identification of *P. ridibundus* and *P. lessonae* genomes in spermatids of diploid hybrid male. Using a combination of both probes, specific to *P. ridibundus* chromosomes (*RrS1*, indicated by arrowheads) and *P. lessonae* chromosomes (*PlesSat01-48*, coded as Ples289 in the Figure and indicated by arrows) it is possible to identify spermatids with only *P. ridibundus* genome (bottom right) and only with *P. lessonae* genome (top left) simultaneously produced by diploid hybrid male. Scale bars = 10 μ m.



Supplementary Figure S3. FISH-based mapping of interstitial tandem repeat PlesSat02-200 on chromosomes of *P. ridibundus*, *P. lessonae* and diploid *P. esculentus*. Tandem repeat *PlesSat02-200* (coded as Ples385 in the Figure) localised in interstitial site of long arms on chromosome 6/7 (see the main text) of *P. ridibundus* (a), *P. lessonae* (b), and *P. esculentus* (c), (indicated by arrows). In *P. lessonae*, it also occupies short arms of chromosome 6/7 and additionally short arms of chromosome 4 (indicated by arrowheads). In *P. esculentus*, we detected both bright and weak signals on homologs of chromosome 6/7 and weak signals on one homolog of chromosome pair 4. Scale bars = 10 μ m.