

## Testing skin swabbing for DNA sampling in dendrobatid frogs

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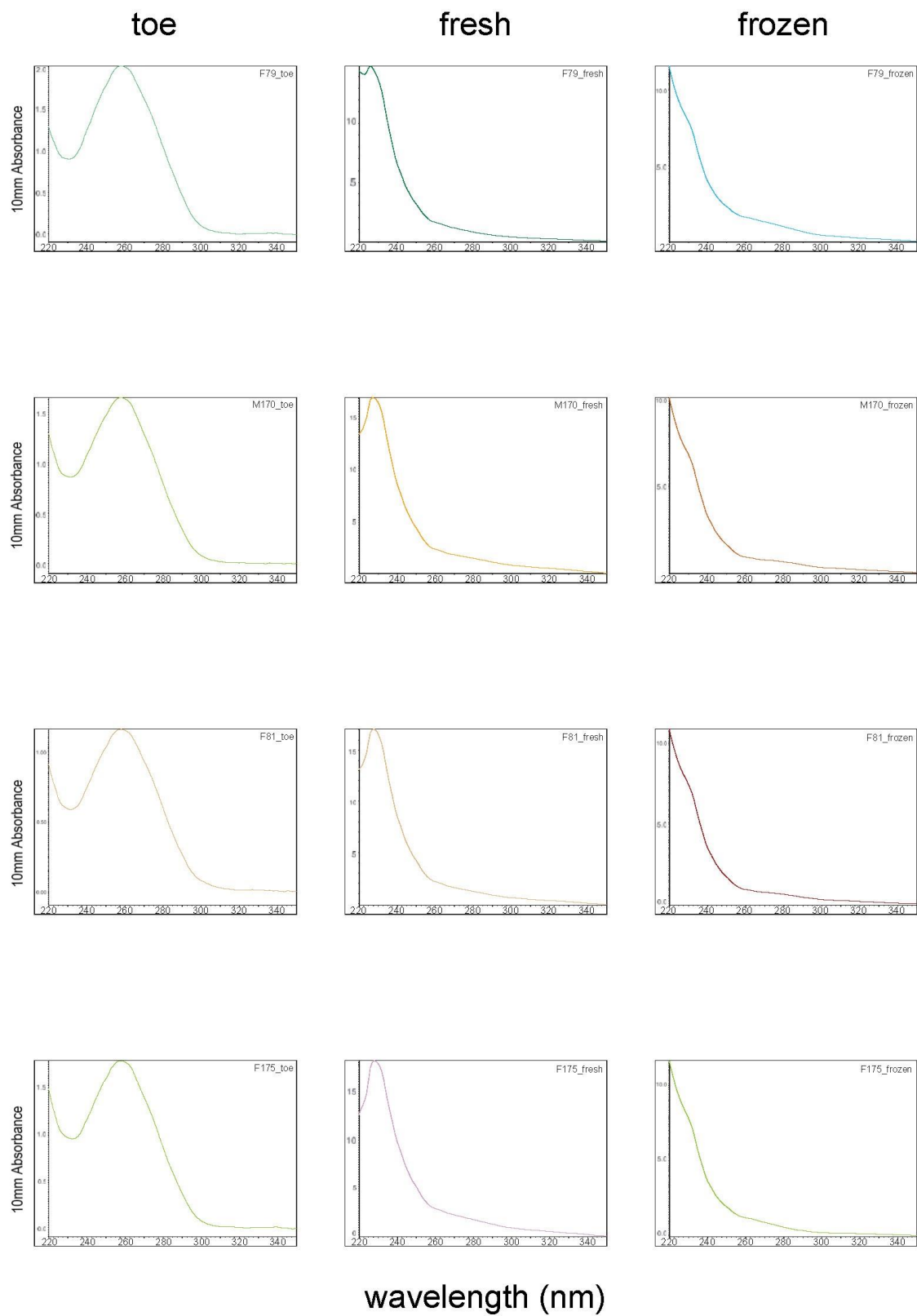
### **Supplementary material**

#### *Genotyping*

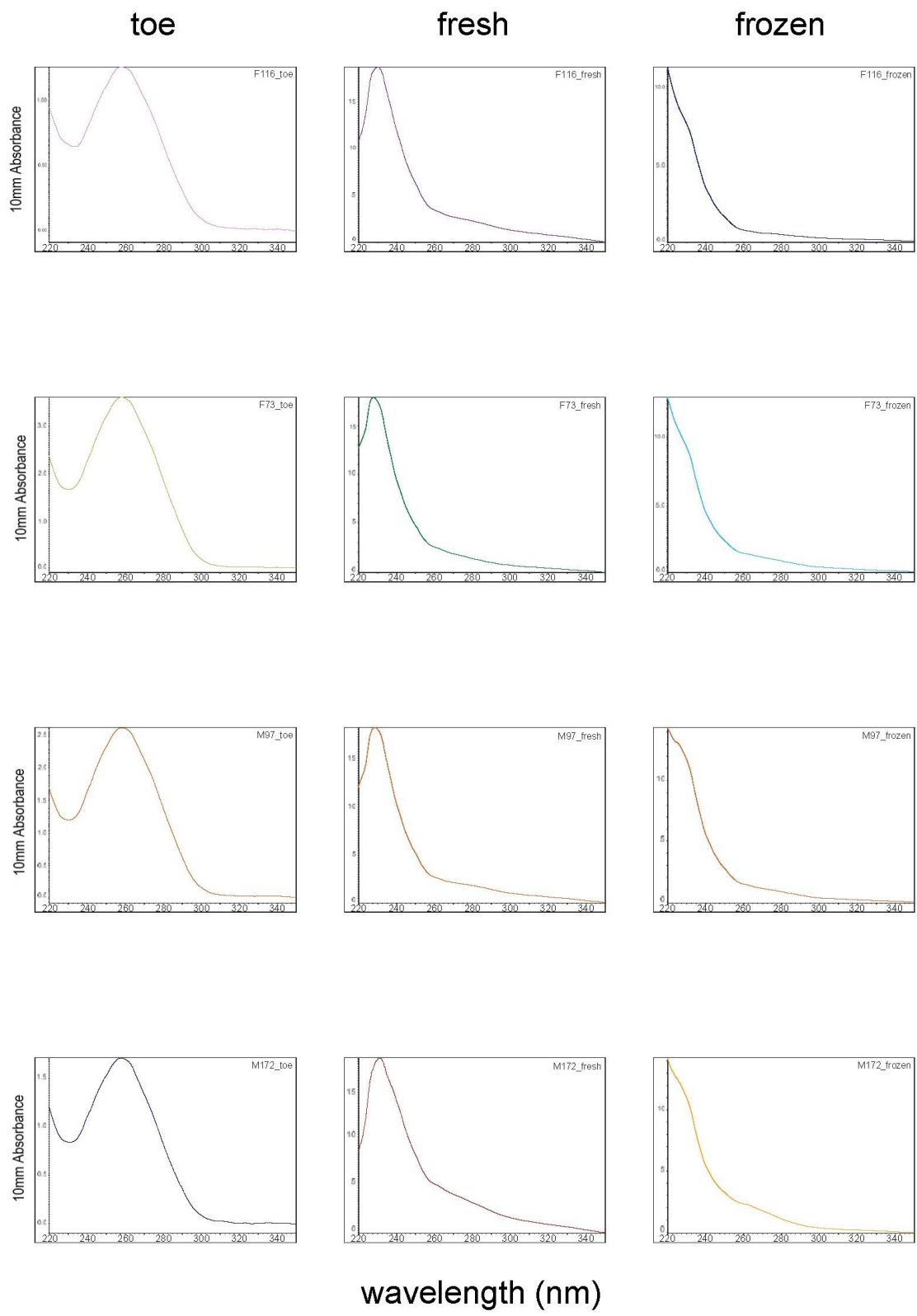
PCR amplifications were performed using reaction volumes of 10 µl containing 10 ng of genomic DNA, 0.2 mM of each dNTP, 1 µM of each forward and reverse primer, 0.5 U of Taq DNA polymerase (Axon), 1 µl of 10×NH<sub>4</sub> reaction buffer (Axon), at a final concentration of 1.5 mM MgCl<sub>2</sub>. Finally, we added 0.1 µl of Bovine Serum Albumin to each reaction. We used the following PCR program: 5 min at 95 °C, 35 cycles at 95 °C for 45 s, the primer specific annealing temperature (Table S1) for 45 s, 72 °C for 45 s, followed by a final extension step for 5 min at 72 °C. Differences in the sizes of the amplified products and in the fluorescent dyes of the primers allowed for pooling of multiple loci in the subsequent sequencing process. The pooled products were diluted with water 1:20, mixed with HiDiformamid and the internal size standard ROX500 (Applied Biosystems), and run on an ABI 3130xl Genetic Analyzer. Alleles were manually extracted with Peakscanner software (Applied Biosystems), and final allele sizes were determined using TANDEM v.1.08 (Matschiner & Salzburger, 2009). PCRs were repeated up to three times in case of low amplification success.

#### **References**

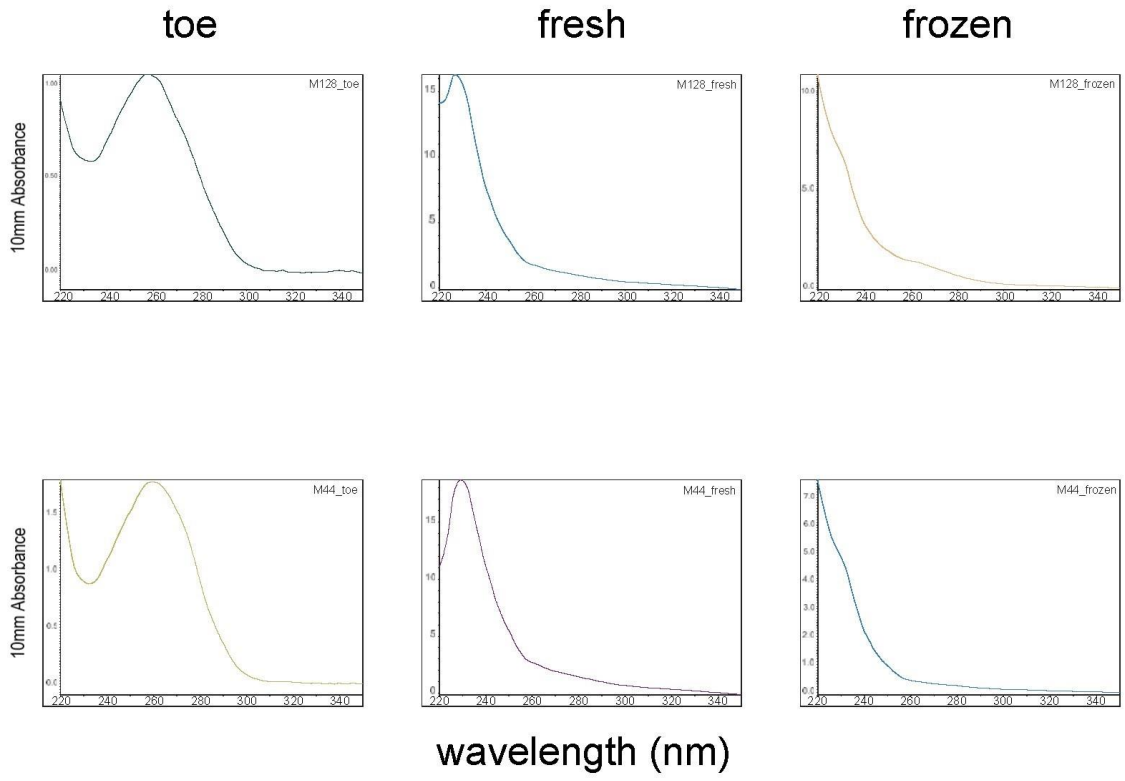
Matschiner M, Salzburger W (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* **25**: 1982-1983.



**Figure S1.** Spectral curves of all samples used in this study.



**Figure S1.** Continued.



**Figure S1.** Continued

**Table S1.** Details on the 11 microsatellite loci used in this study.

Locus	Dye and primer sequence (5'–3')	AT (°C)	GenBank accession no
<i>Afem03</i>	F: (FAM)-ATGAACACAGAGCCGAGACC R: TCAATATGTCAATGTATCTTTCAATC	58	EU414774
<i>Afem04</i>	F: HEX-GAGACGCCTGTTATAGATGGTG R: TTAAAATGCACCGACTGG	56	KF021569
<i>Afem09</i>	F: (TET)-TTCCCAACATCCCTACATCC R: TCAATTCACAGCACCCTCC	57	EU414777
<i>Afem12</i>	F: (FAM)-GGCCTTTGTGACATGTGATG R: CTCCAGGCCTCAAACAAGAG	58	EU414778
<i>Afem13</i>	F: (FAM)-TTTGTAGGTGGGAAAACCTTGC R: TCAGCTTCCAAGAATAAATACGG	58	EU414779
<i>Afem15</i>	F: (HEX)-GAATATTCAGTGTCCCGGAAG R: GTGCCGCTCATTAAGCTCAT	56	EU414781
<i>Afem16</i>	F: (HEX)-ACAGGGTAGAGGCTTTGGTG R: TCATTGAGGTCTTAGTTTTCCA	56	EU414782
<i>Afem20</i>	F: TET-TCCTAAGCCAGAGGAAGCTG R: ACAATCACATGCACCGAGTC	56	KF021570
<i>Afem22</i>	F: NED-ACCGTGGAGTGGTTGATGAG R: CCTGCCAAGGATTGATAAGC	52	KF021571
<i>Afem24</i>	F: NED-AAAGTAGGGTCGCAGCACTC R: AGGTCAAGTCGGATGGTTTG	56	KF021573
<i>Afem25</i>	F: HEX-GTAATCCCCCAATCCTGGTC R: GATCCCGGCATCGTTAAG	61	KF021575

**Table S2.** KS-test. Significant deviation from normal distribution is given in bold.

	<i>n</i>	$X^2$	<i>P</i>
Quantity (ng/μl)	30	0.144	0.114
260/280	30	0.096	0.1
<b>260/230</b>	30	0.377	<b>&lt; 0.001</b>
<b>Amplification success</b>	30	0.215	<b>0.001</b>

**Table S3.** Results of the Kruskal-Wallis tests and corresponding post-hoc Mann-Whitney U tests

		Mann-Whitney <i>U</i> test	Kruskal-Wallis test
Quantity	toes vs fresh	$U = -7.6$ adj. $p$ -value = 0.161	
	toes vs frozen	$U = 7.3$ adj. $p$ -value = 0.191	$H_2 = 14.325$ $p = 0.001$
	fresh vs frozen	$U = 14.9$ adj. $p$ -value < 0.001	
260/280	-	-	$H_2 = 5.784$ $p = 0.055$
260/230	toes vs fresh	$U = 14.5$ adj. $p$ -value = 0.001	
	toes vs frozen	$U = 15.5$ adj. $p$ -value < 0.001	$H_2 = 19.467$ $p < 0.001$
	fresh vs frozen	$U = 1$ adj. $p$ -value = 1	
AS	toes vs fresh	$U = 17.35$ adj. $p$ -value < 0.001	
	toes vs frozen	$U = 12.65$ adj. $p$ -value = 0.01	$H_2 = 21.272$ $p < 0.001$
	fresh vs frozen	$U = -4.7$ adj. $p$ -value = 0.681	