### DNA Extraction Sri Bulan Musmiah G352110101

#### EKSTRAKSI DNA

* Sri Bulan Musmiah G352110101

<table>
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<td>Mitochondrial control region Total genome</td>
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<td>Fresh dung (pellets or portions of dung patties) was collected by observing focal individuals until the subject defecated. Samples were collected in a clean resealable bag. These bags were kept in an iced cooler and transported back to lab within 24 h, then stored at -20°C. Frozen dung samples were cut using a sterile scalpel, and approximately 0.25 g of dung was teased out from the middle of several freshly cut subsamples.</td>
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DNA was extracted using the UltraClean® Fecal DNA isolation kit rinsed twice in DNase/RNase-free distilled water. After decontamination, two different DNA-extraction techniques were applied. The samples first underwent an MSSC extraction protocol designed for degraded DNA samples. As keratin is the major structural component of feather barbs, we modified the lysis buffer to include dithiothreitol (DTT). Barbs were incubated in 1.5 to 3 ml of lysis buffer (0.05 mol/l EDTA pH 8.0, 0.5% SDS, 0.5 mg/ml proteinase K and 10 mg/ml DTT) at 55°C for 1 to 2 hours. The remainder of the DNA extraction producing a final elution of 100 µl of DNA solution for each sample. Subsequently, the feather-barb subsamples were also extracted using a commercial kit producing a final elution of 100 µl of DNA solution for each sample.

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Individuals with opened and closed shells were preserved from each beach in 80% ethanol. Muscle tissue was extracted from the middle and apex region (c. 1 mm$^2$) of the foot and cleaned with ethanol (75%) to remove sand, detritus or external organic matter. DNA extraction was performed with the Qiagen DNMi kit, with slighty modification to increase the concentration of DNA, i.e. we used 150 µl of elution buffer.
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<th>Achmad Farajallah</th>
<th><a href="mailto:achamad@ipb.ac.id">achamad@ipb.ac.id</a></th>
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Egg yolks, slough skin and non-invasively sampled (NIS) muscle, blood, and faecal material.
Sloughed skins, mostly collected at the entrance of identified adder domains, were preserved dry at room temperature for up to two years. An additional fresh slough was obtained from an adder observed in the process of ecdysis. Snake faeces (associated with sloughs) collected from a range of UK sites were either immediately frozen at -20°C or preserved in 95% ethanol. A 10 µl blood sample, obtained by caudal extraction from an adder and stored in 90 µl of Seutin's buffer (Seutin et al., 1991) at room temperature, was collected as a positive PCR control. Sloughed skin required a rehydration step to remove impurities prior to DNA extraction. A fragment (1-2 cm²) of slough was rinsed in 1 ml of ddwater at 55 °C in a rocking incubator for 4-6 h, and repeated for a further 8-12 h. DNA extraction was performed on these rehydrated samples, egg yolks (approximately 0.2 cm³), NIS muscle (1 cm³) and blood (5 µl in Seutin's buffer) using QiagenDNeasy tissue extraction kit. Faecal material was extracted using QIAamp® DNA stool mini kit.
The soft tissue, stomach and pyloric caeca.
Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice, and subsequently stored at -20°C. The soft tissue, stomach and pyloric caeca, of each starfish was removed and separated from the skeleton. The DNA was extracted using AxyPrep multi source Genomic DNA Mini-Prep Kit.
Genome
DNA
Leukocytes and finger nails
As a blood sample was available only from the proband, clipped fingernail samples were obtained from 10 of the other 12 members instead. Genomic DNA was extracted from the fingernails using a buffer solution containing urea, DDT and proteinase K, as reported previously. Briefly, clipped fingernails were once frozen in liquid nitrogen and crushed into fine powder using Multi-beads Shocker™ (Yasui Kikai, Osaka, Japan). The nail powder was lysed in a urea-lysis solution (2 M urea; 0.5 % SDS; 10 mM Tris-HCl, pH 7.5; 0.1 M EDTA) containing 1 mg/ml proteinase K and 40 mM DDT at 55 °C overnight. Nail DNA was extracted with phenol/chloroform, and precipitated with ethanol and sodium acetate. Precipitated nail DNA was dissolved again in extraction buffer (0.5 % SDS; 10 mM Tris-HCl, pH 7.5; 0.1 M EDTA) containing 1 mg/ml proteinase K, and incubated at 55 °C overnight. DNA was purified again as above, and was suspended in 30 µl of 1x TE buffer.
Blood and Saliva
The first set DNA, blood of subjects which agreed consent obtained by phlebotomized and shipped to laboratory in Iowa City. The DNA from a 15 ml sodium citrate tube of blood was obtained using the cold protein precipitation method (Lahiri and Nurnberger 1991). The second set of DNA sample which was derived from saliva (SD for saliva derived). DNA from these caretaker-samples was processed using materials and methods using Oragene! kits (DNA Genetek, Canada). Subjects rinsed their mouths, then deposited 4 ml of saliva into the Oragene sample container after receiving instructions from a trained research assistant. Both types of DNA samples were then quantified spectrophotometrically, then stored at -20°C until use.


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<td>DNA extracted of the collected feathers followed bags that had photos of the feathers in them. The feathers were then transported back to lab where they were placed at 50°C to remove any remaining proteinase K and 0.47 M EDTA.</td>
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Note: The table provides information on DNA extraction methods for different species and samples. Each sample type has a specific extraction protocol to ensure the integrity of the DNA extracted.
DNA was extracted using the UltraClean® Fecal DNA isolation kit rinsed twice in DNase/RNase-free distilled water. After decontamination, two different DNA-extraction techniques were applied. The samples first underwent an MSSC extraction protocol designed for degraded DNA samples. As keratin is the major structural component of feather barbs, we modified the lysis buffer to include dithiothreitol (DTT). Barbs were incubated in 1.5 to 3 ml of lysis buffer (0.05 mol/l EDTA pH 8.0, 0.5% SDS, 0.5 mg/ml proteinase K and 10 mg/ml DTT) at 55°C for 1 to 2 hours. The remainder of the DNA extraction producing a final elution of 100 µl of DNA solution for each sample. Subsequently, the feather-barb subsamples were also extracted using a commercial kit producing a final elution of 100 µl of DNA solution for each sample.

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