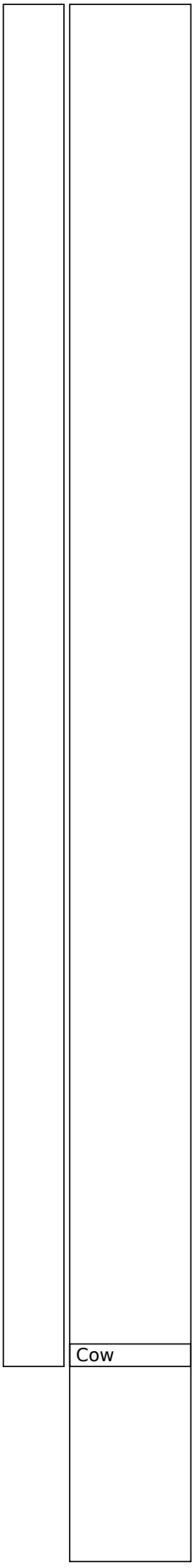
DNA Extraction Sri Bulan Musmiah G352110101

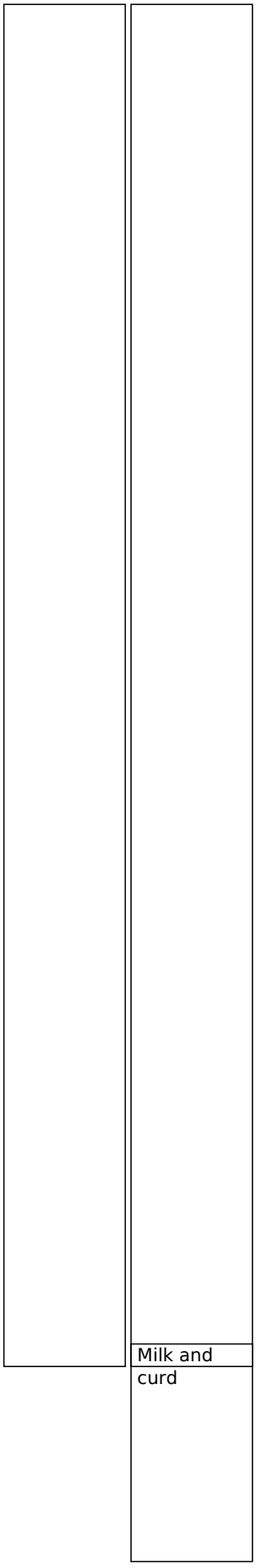
EKSTRAKSI DNA
Sri Bulan Musmiah G352110101

NO	SPECIES	SAMPLE	DNA EXTRACTION	GENOME TARGET
1	Moa(Aves: Dinornithiformes)	Bone powder	DNA was extracted from 200 mg of bone powder by incubation with rotation at 55°C for 48 h in 1.5 mL digestion buffer [20 mM Tris, pH 8.0, 1% Triton X-100, 10mM dithiotheitol (DTT), 1 mg/mL proteinase K and 0.47 M EDTA].The supernatant was spun through 30,000 MWCO Vivaspin columns and then combined with 5 volumes of PBI buffer (Qiagen, Valencia, CA) before the DNA was extracted using silica spin columns (Qiagen).	Mitochondrial control region Total genome
2	Cows,prong horn, bison , black-tailed prairie dogs (Fungi?)	Dung	Fresh dung (pellets or portions of dung patties) was collected by observing focal individuals until they 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. DNA was extracted using the UltraClean® Fecal DNA isolation kit	Fungal total genome
3	Birds	Feather	DNA extraction of the archaeological feather followed the same protocols as the fresh samples. The protocols follows strict contamination-control for the analysis of ancient remains. Two and five feather barbs were removed from the feather shaft. Barbs were first rinsed in 3% sodium hypochlorite for 30 seconds to remove possible surface contamination, then 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 keratinis 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	~~Fragments 200 to 300 bp in size of the mitochondrial DNA cytochrome b gene~~ Total genome - fragmented genome

commercial kit producing a final elution of 100 µl of DNA solution for each sample.

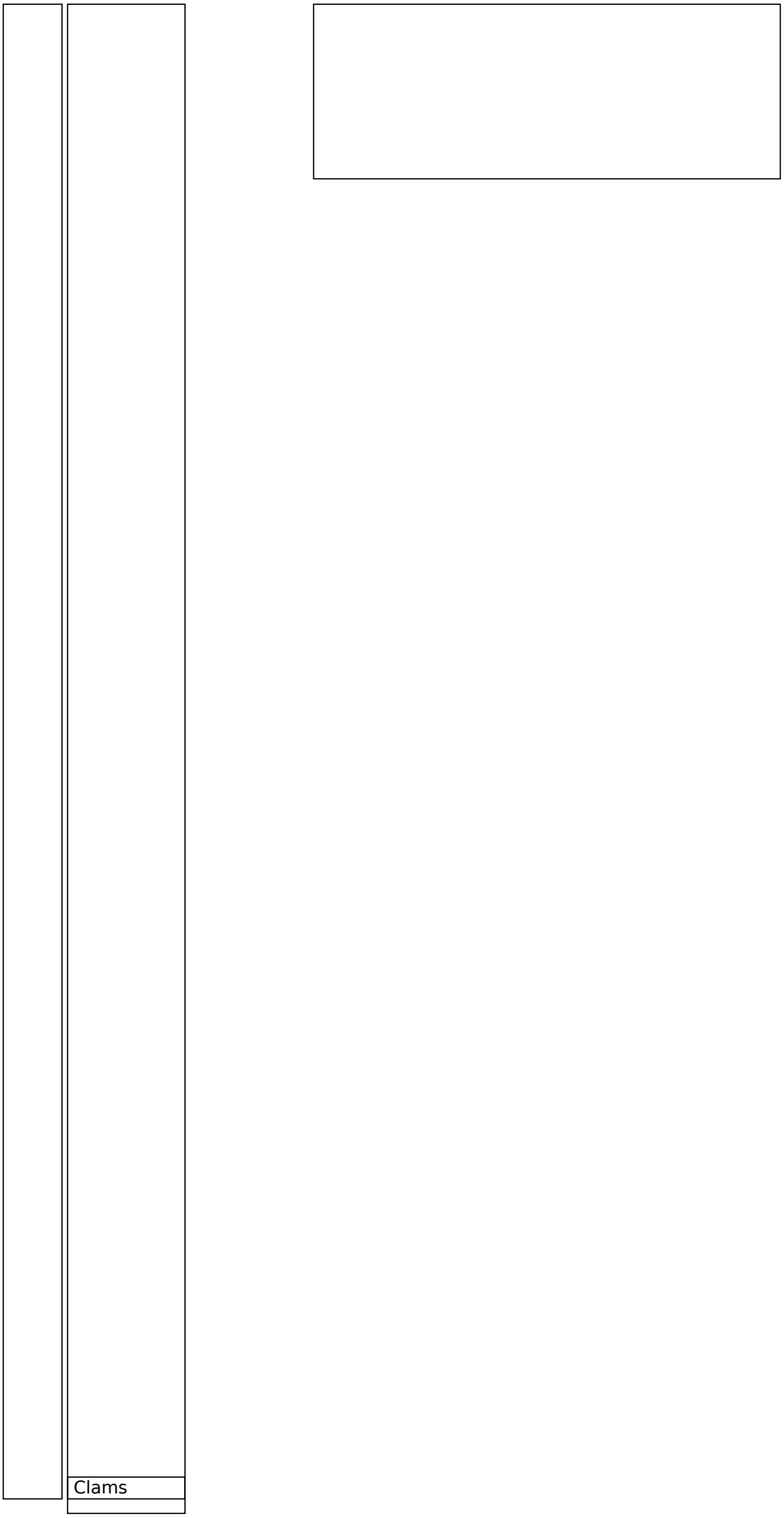
4


Cow


Milk and
curd

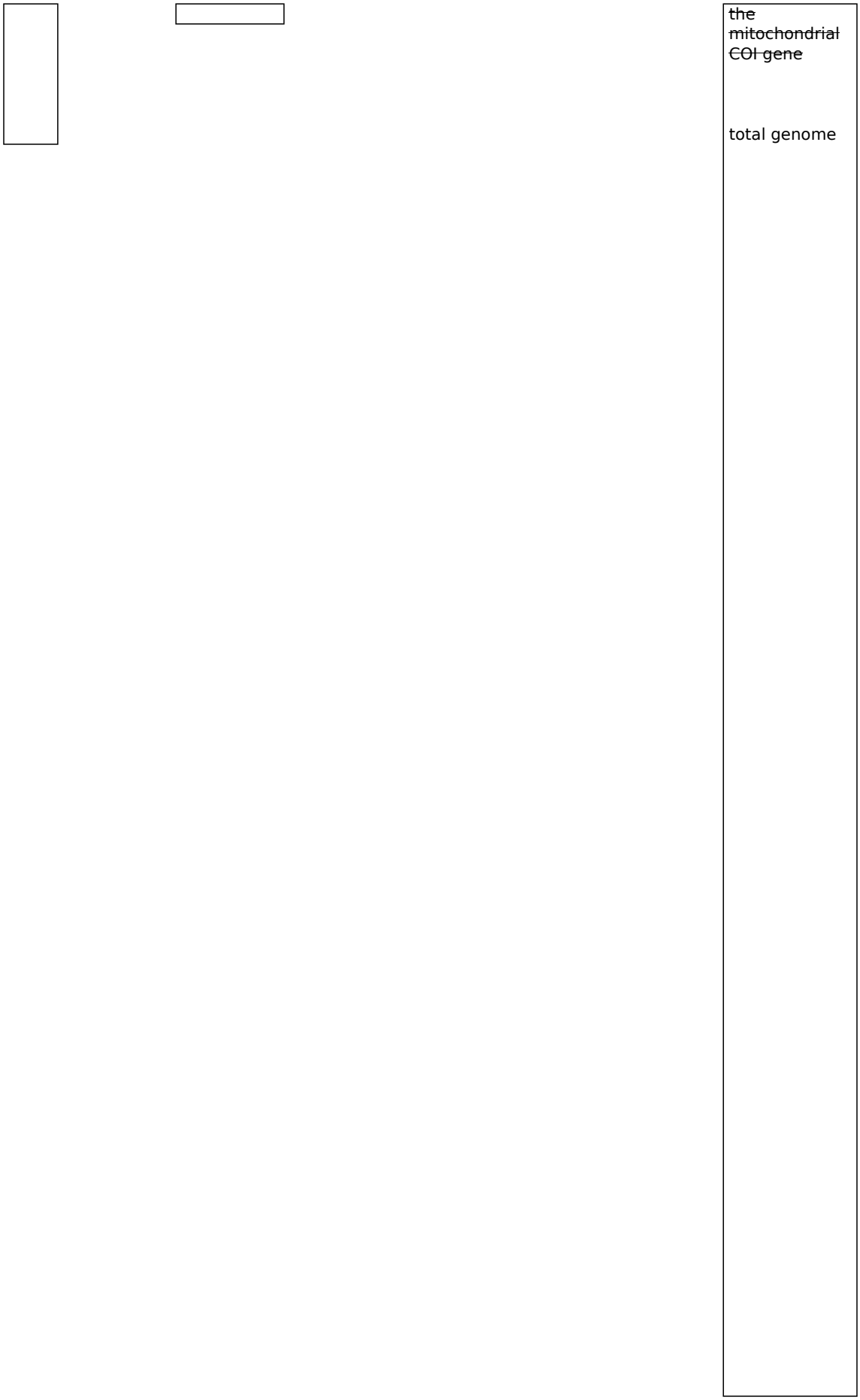
	Total DNA was isolated from milk samples by a phenol/chloroform method, followed by ethanol precipitation according to Sambrook et al.(1989)Cheese samples were cut into small pieces, and total DNA from cheese matrix was obtained by performing	Total genome, include microbial genom

	phenol/chlorophorm as described above. DNA from reference strain of bacteria were prepared from pure culture according to the guanidium extraction procedure described by Pitcher et al. (1989).
5	


Clams

	Muscle	Individuals with opened and closed shells were

tissue	preservedfrom each beach in 80% ethanol. Muscle tissue was extracted from the middle and apex region (c. 1mm^2) of the foot and cleaned with ethanol (75%) to remove sand, detritusor external organic matter.DNA extraction was performed with the Qiagen DNAMini kit, with slighlty modification to increase the concentration of DNA, i.e. we used 150 µl of elution buffer.	
		~~Fragment of~~



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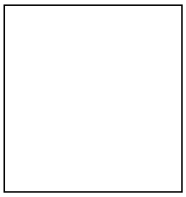
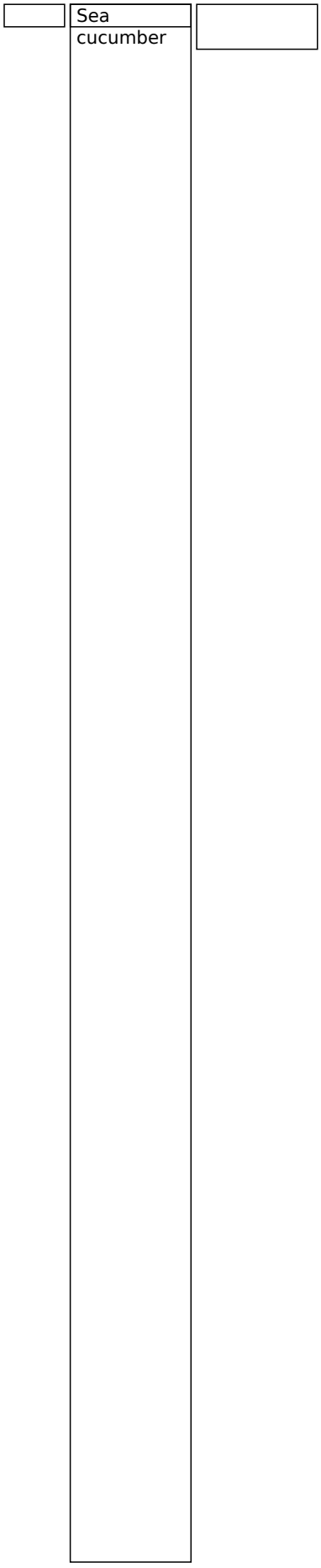
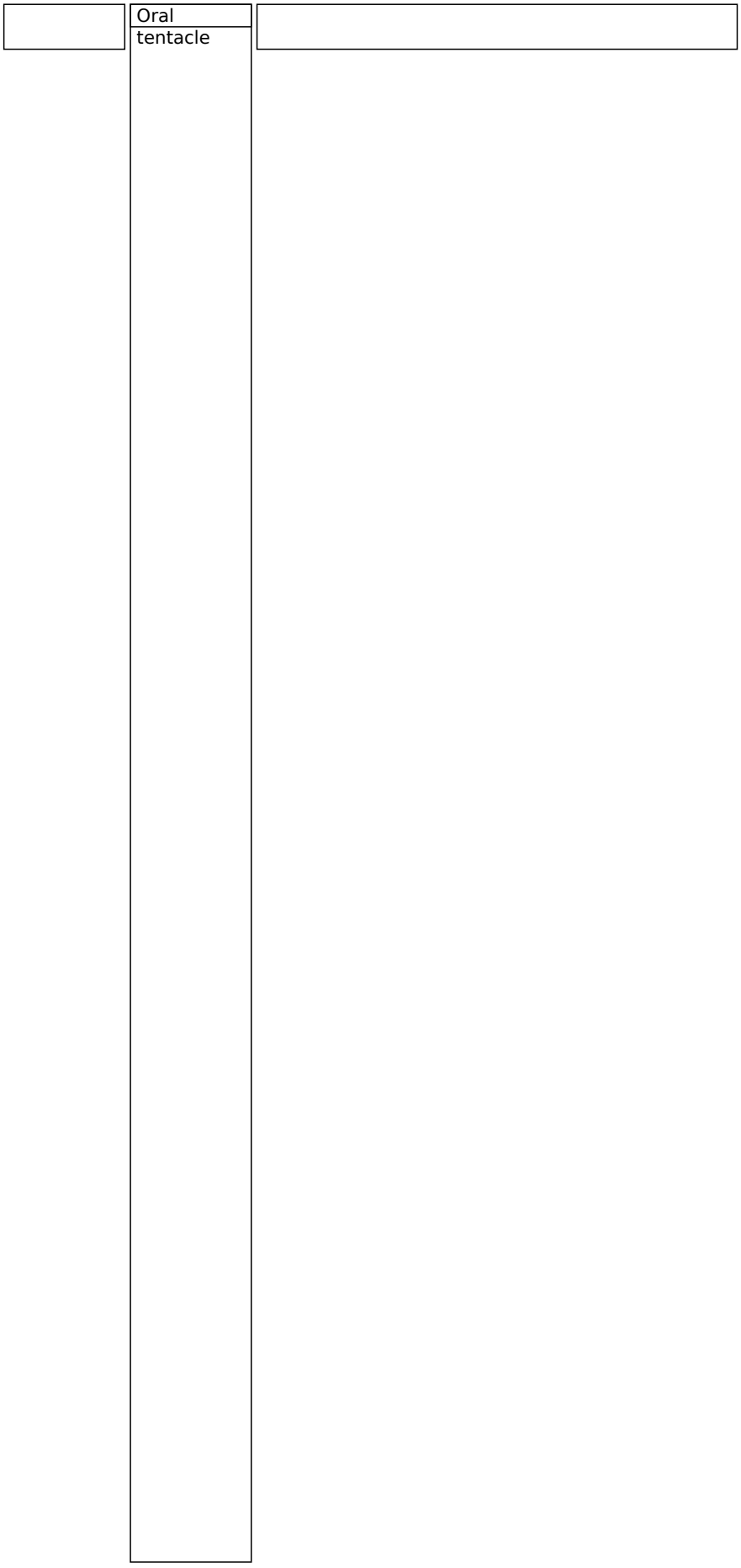
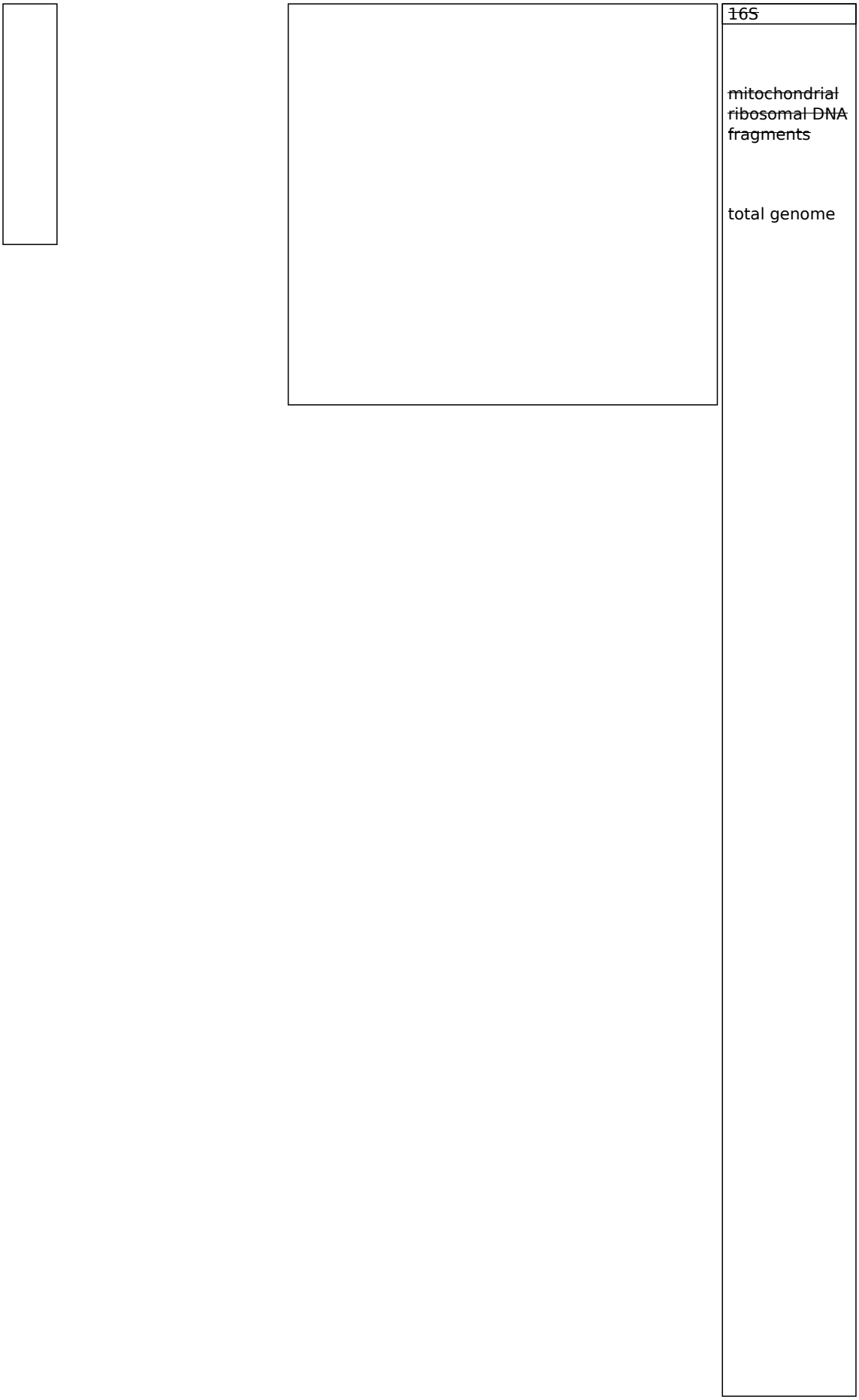


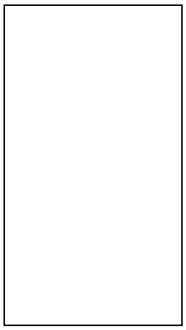

Sea
cucumber

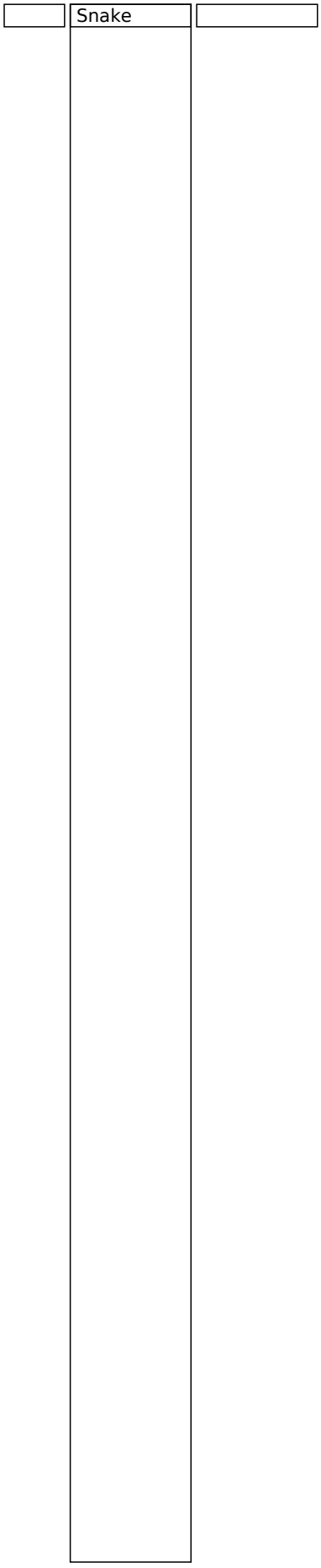

Oral
tentacle

	Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice (-70°C). Typically 200 mg of oral tentacle was ground in liquid N2 in the presence of 600 µl of proteinase solution (50 mM Tris–HCl, pH 7.5, 50 mM EDTA, pH 8.0, 0.4% SDS, 0.5 mg/ml Proteinase K). The ground samples were immediately placed at 65°C for minimum of 2 h. The digested samples were repeteadly extracted with phenol:chloroform:isoamyl alcohol, 25:24:1. The resultant aqueous phase was adjusted to 0.5 M NaCl and to 1% cetyltrimethylammonium bromide (CTAB) and incubated for a further 20 min at 65°C. After two final phenol/chloroform extractions, total DNA was precipitated by adding an equal volume of isopropanol followed by sedimentation at 13,000 rpm for 20 min. The DNA pellet was rinsed with 500 µl of 70% ethanol, air-dried, and resuspended in 200 µl of TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA).	


~~16S~~
~~mitochondrial ribosomal DNA fragments~~
total genome

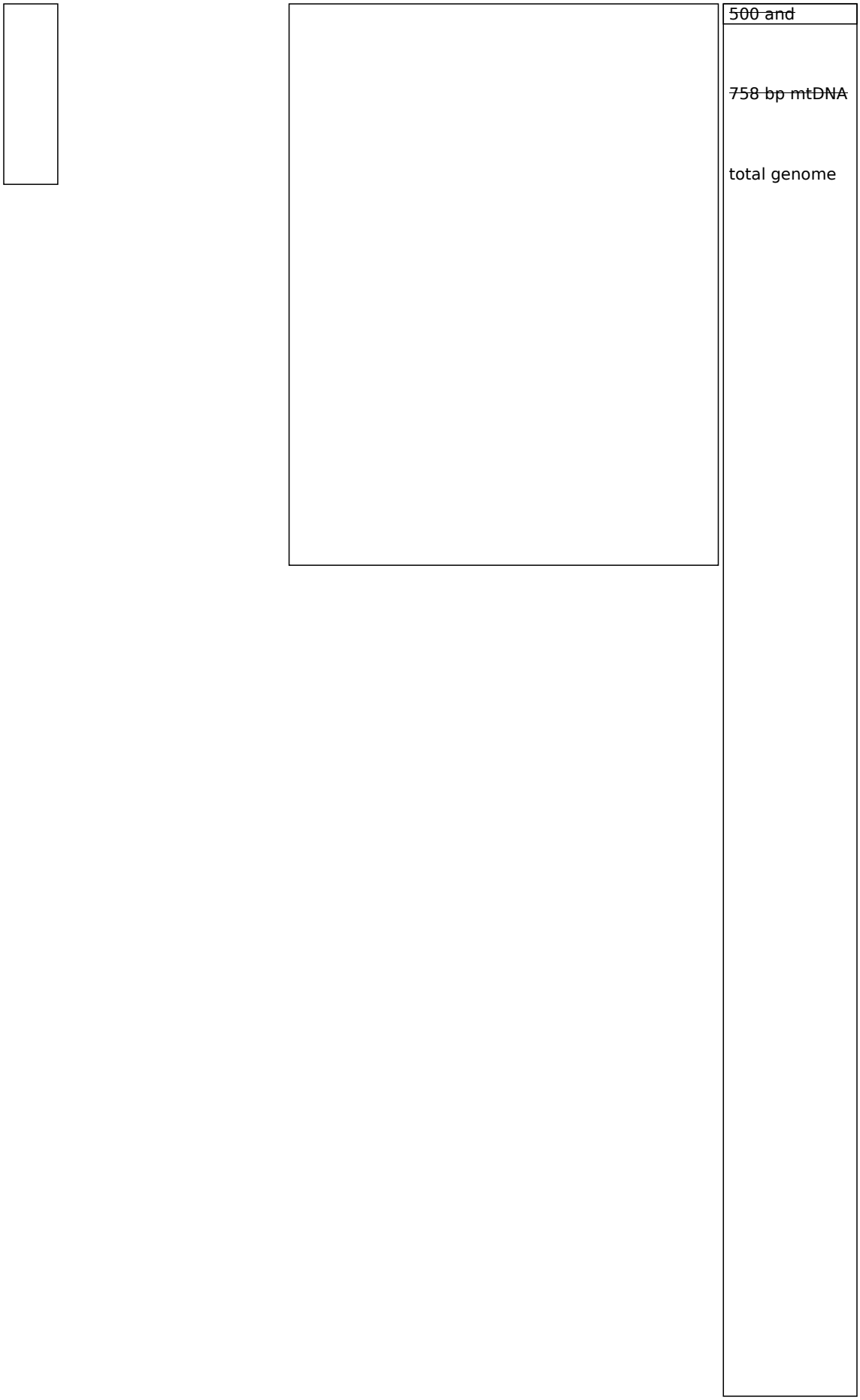
7	



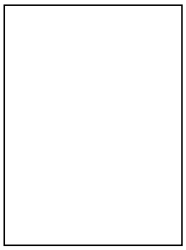

Snake

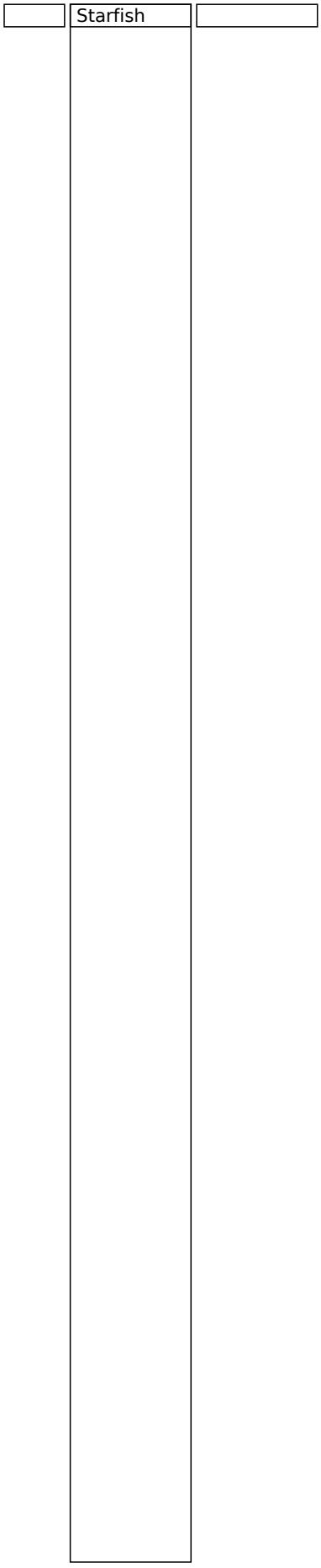
	Egg yolks, slough skin and non-invasively sampled (NIS)muscle, blood, and faecal material.	

	Field collected carcasses, road kills and foetal samples were directly frozen at -20'C or preserved in 95% ethanol. Ancient ethanol preserved tissue (A.E.P.T.; collected pre-1907 to 1969) consisted of museum samples. Non-viable grass snake eggs,located in compost heaps, were preserved at -20^{0}C. 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 -20ºC 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 cm^2) of slough was rinsed in 1 ml of ddwater at 55 ºC 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 cm^3), NIS muscle (1 cm^3) and blood (5 µl in Seutin'sbuffer) using QiagenDNeasy tissue extraction kit. Faecal material was extracted using QIAamp@ DNA stool mini kit.	


~~500 and~~
~~758 bp mtDNA~~
total genome

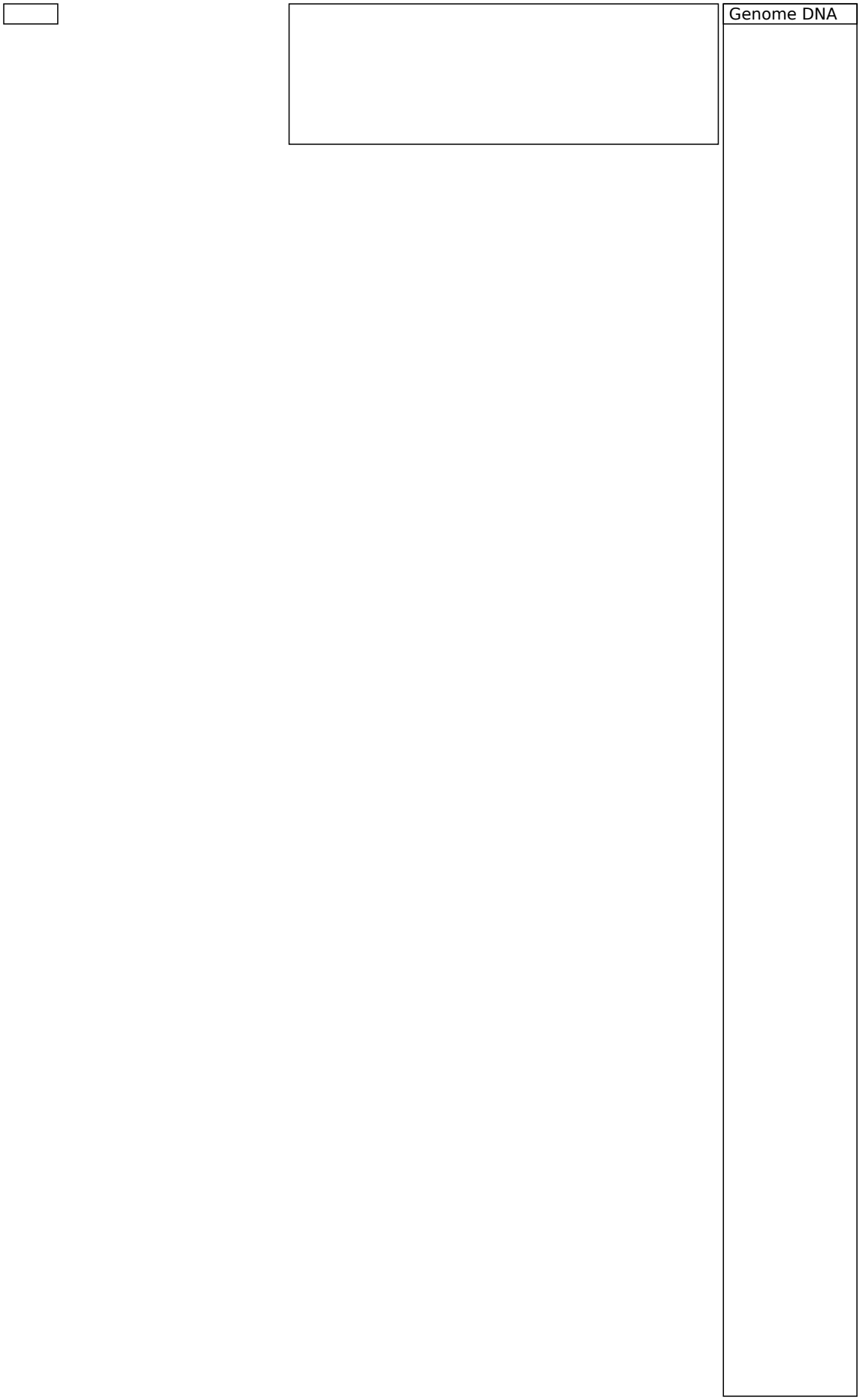
8





	The soft tissue, stomach and pyloric caecae	

	Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice, and subsequently storedat -20°C. The soft tissue, stomach and pyloric caecae, of each starfish was removed and separatedf rom the skeleton. The DNA was extracted using AxyPrep multi source Genomic DNA Mini-Prep Kit.	

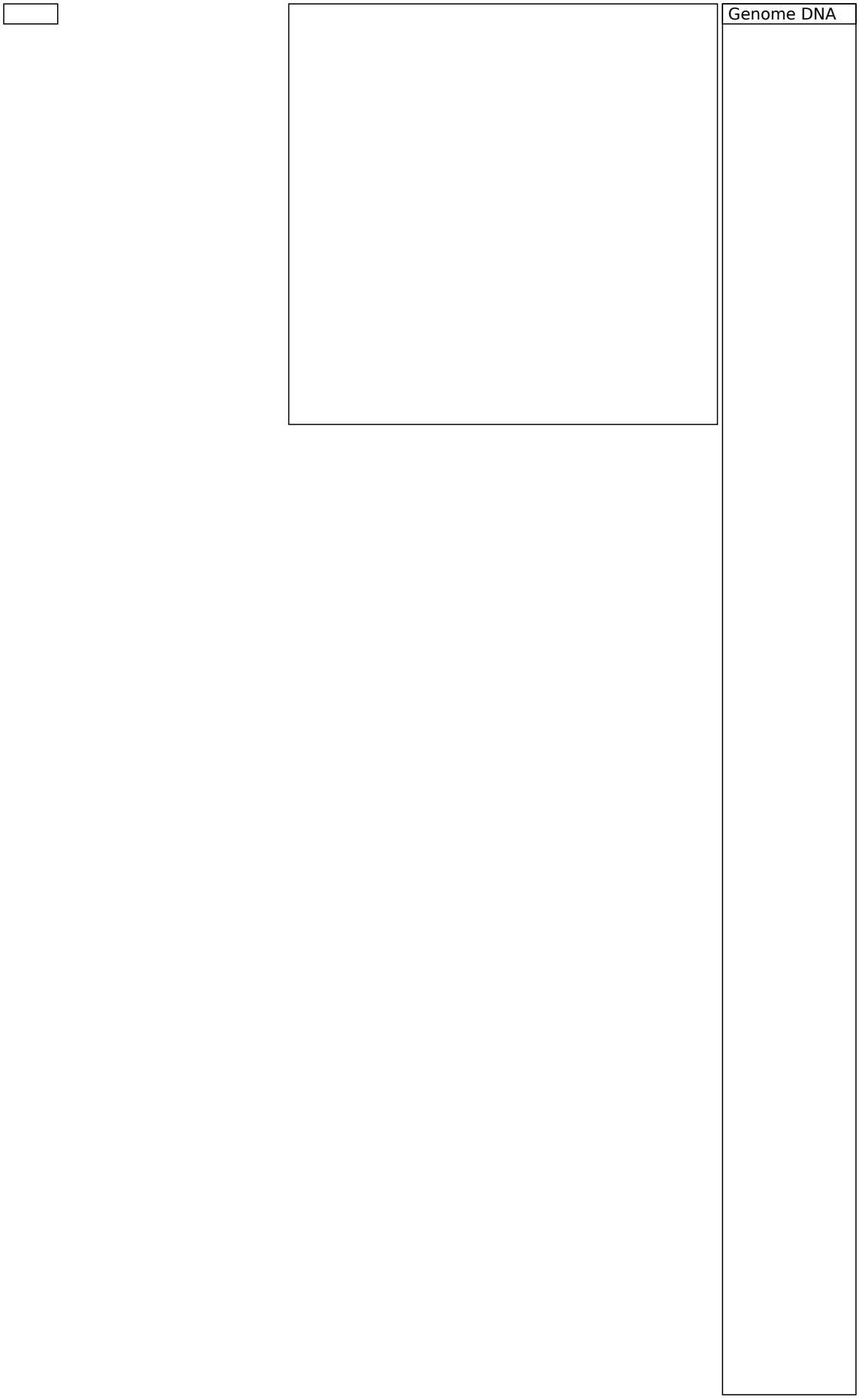


9

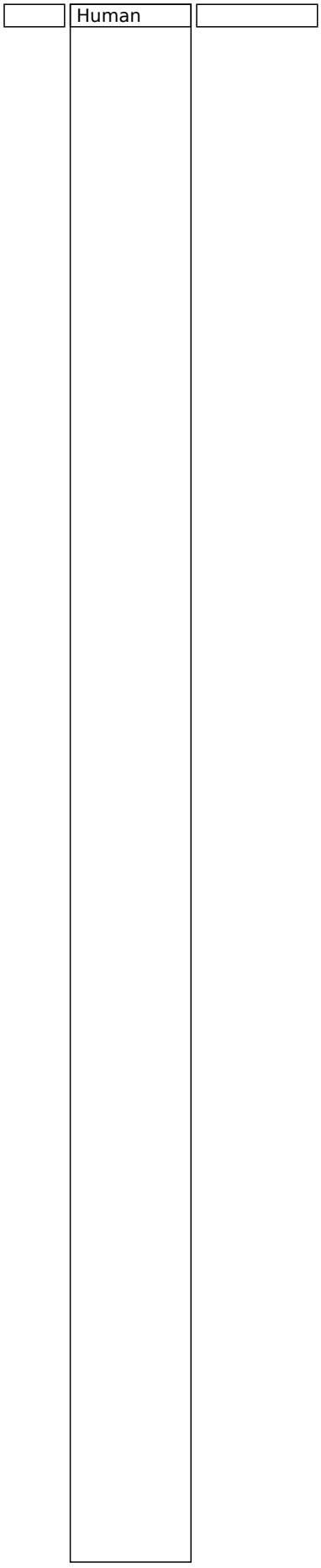
	Human	

	Leukocyt-es 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 purifiedagain as above, and was suspended in 30 µl of 1x TE buffer.	


Genome DNA

10		



	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.	

		Genome DNA

1 Allentoft, M.E. , Bunce, M. , Scofield, R.P., Hale, M.L. and Holdaway, R.N. 2010. Highly skewed sex ratios and biased fossil deposition of moa: ancient DNA provides new insight on New Zealand's extinct megafauna. Quaternary Science Reviews, 29 (5-6). pp. 753-762.

2 Herrera, J. Poudel, R., and Khidir, H. H. 2010. Molecular characterization of coprophilous fungal communities reveals sequences related to root-associated fungal endophytes. Microb Ecol (2011) 61:239-244.

3 Speller, C.F., Nicholas G. P., and Yang D. Y. 2011. Feather barbs as a good source of mtDNA for bird species identification in forensic wildlife investigations. Investigative Genetics 2011, 2:16.

4 Giannino, M. L., Marzotto, M., Dellaglio F., and Feligini, M. 2009. Study of microbial diversity in raw milk and fresh curd used for Fontina cheese production by culture-independent methods. International Journal of Food Microbiology 130 (2009) 188-195.

5. Carstensen, D., Laudien J., Leese., Arntz W., and Held, C. 2009. Genetic variability, shell and sperm morphology suggest that the surf clams Donax marincovichi and D. Obesulus are one species. Journal of Molluscan Studies Advance Access published 25 September 2009.

6. El-Naggar A.M., Ashaat N. A., El-Belbasi H. I., and Slama M. S. 2008. Molecular phylogeny of egyptian sea cucumbers as predicted from 16s mitochondrial rRNA gene sequences. World Applied Sciences Journal 5 (5): 531-542, 2008.

7. Jones, R., Cable, J., and Bruford M. W. 2008. An evaluation of non-invasive sampling for genetic analysis in northern European reptiles. Herpetological Journal 18 : 32-39, 2008.

8. El- Naggar, A. M. 2009. DNA fingerprinting of some mediterranean and red sea starfish genomes. Research Journal of Cell and Molecular Biology, 3(1): 3

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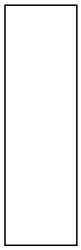
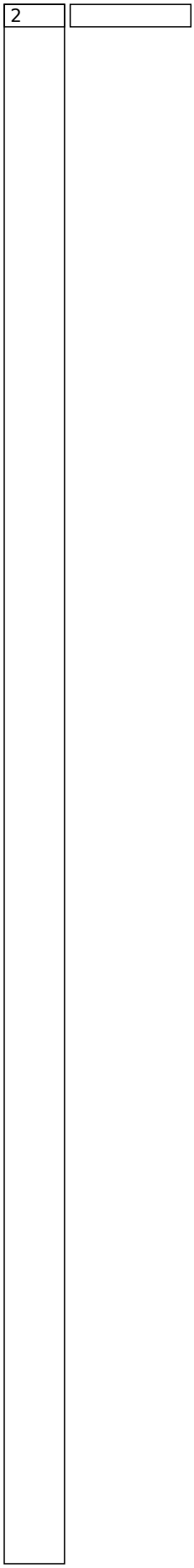
NO	SPECIES	SAMPLE	DNA EXTRACTION	GENOME TARGET
1	Moa(Aves: Dinornithiformes)	Bone powder	DNA was extracted from 200 mg of bone powder by incubation with rotation at 55°C for 48 h in 1.5 mL digestion buffer [20 mM Tris, pH 8.0, 1% Triton X-100, 10mM dithiotheitol (DTT), 1 mg/mL proteinase K and 0.47 M EDTA].The supernatant was spun through 30,000 MWCO Vivaspin columns and then combined with 5 volumes of PBI buffer (Qiagen, Valencia, CA) before the DNA was extracted using silica spin columns (Qiagen).	Mitochondrial control region Total genome

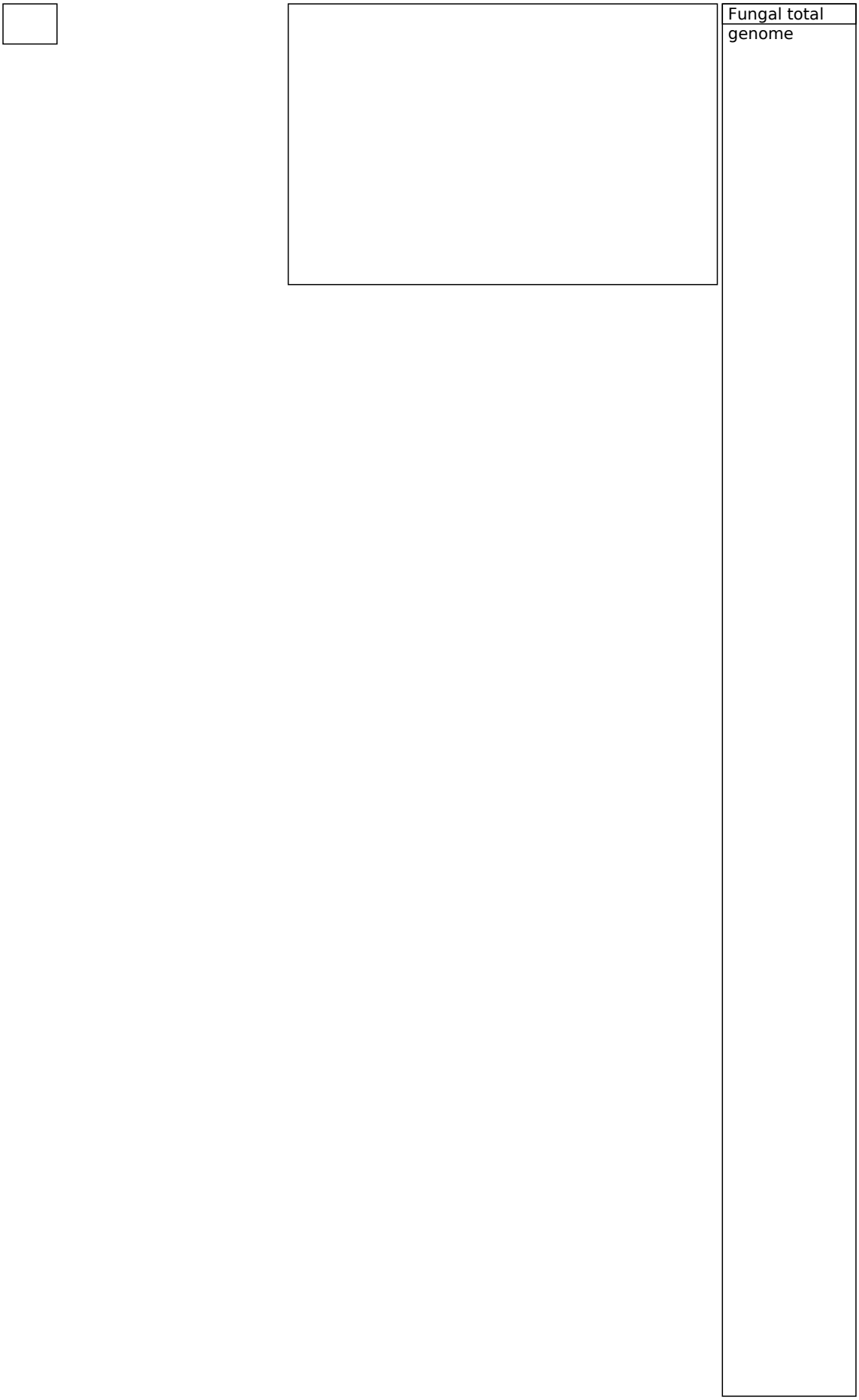


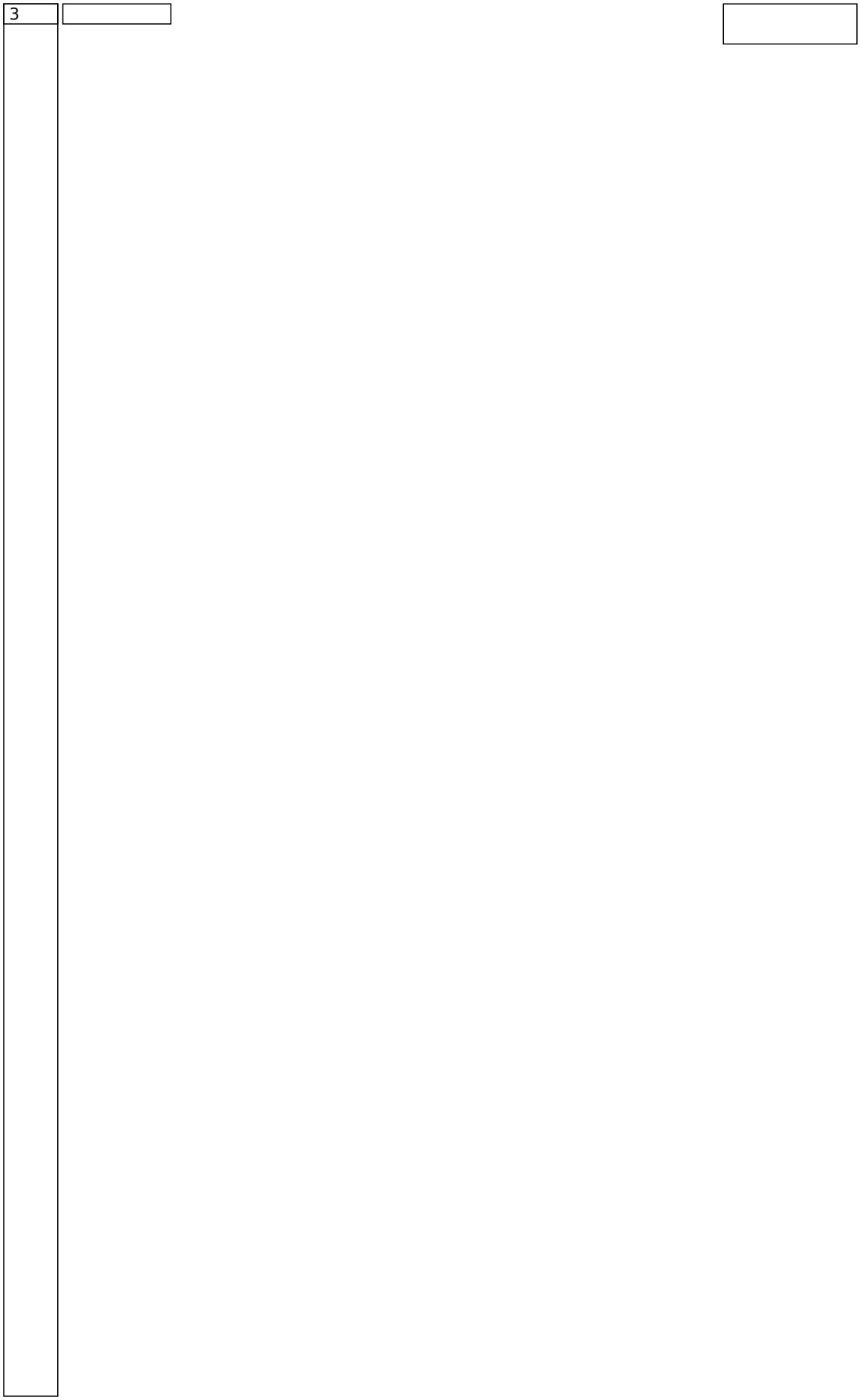

2

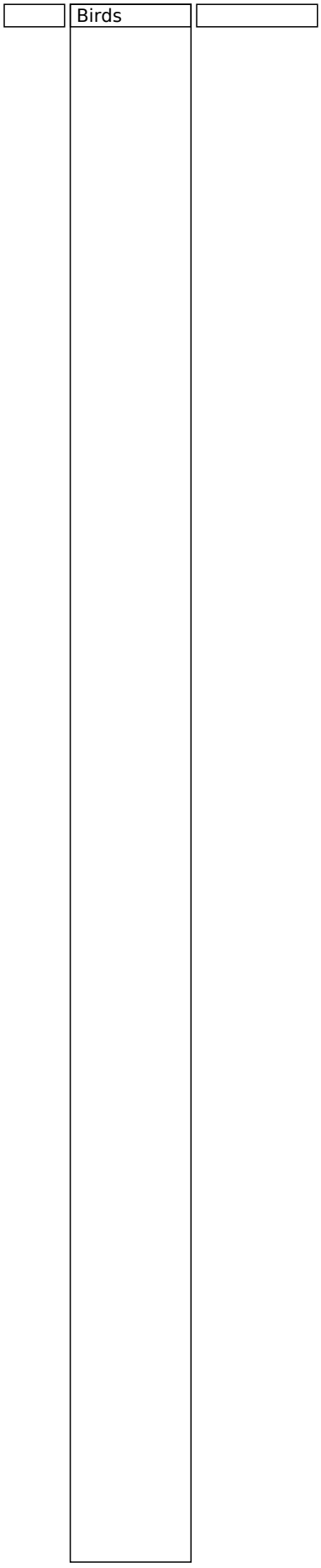
	Cows,prong horn, bison , black-tailed prairie dogs (Fungi?)	

	Dung	

	Fresh dung (pellets or portions of dung patties) was collected by observing focal individuals until they 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. DNA was extracted using the UltraClean® Fecal DNA isolation kit	


Fungal total
genome





	Feather	

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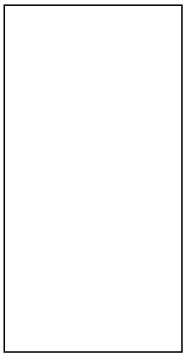
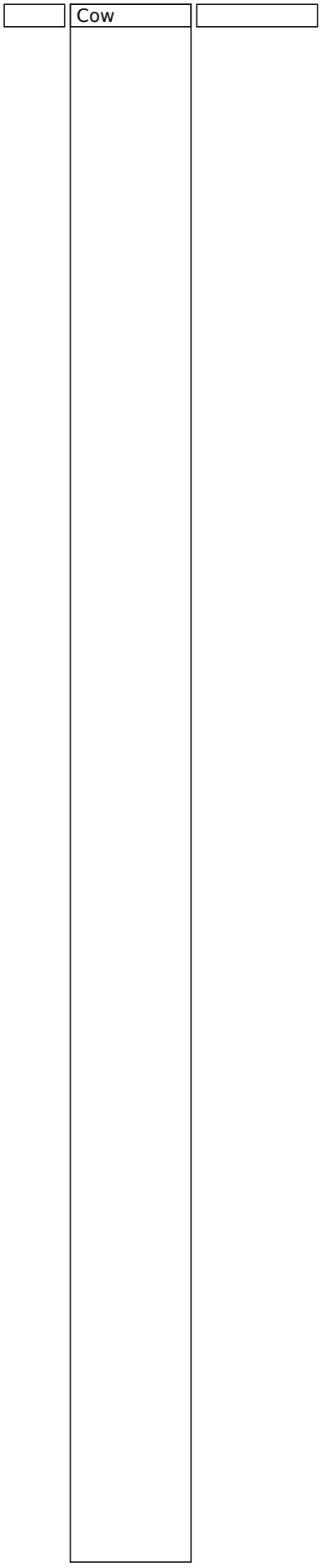
		~~Fragments 200 to 300 bp in size of the mitochondrial DNA cytochrome b gene~~ Total genome - fragmented genome

4	





	Milk and curd	

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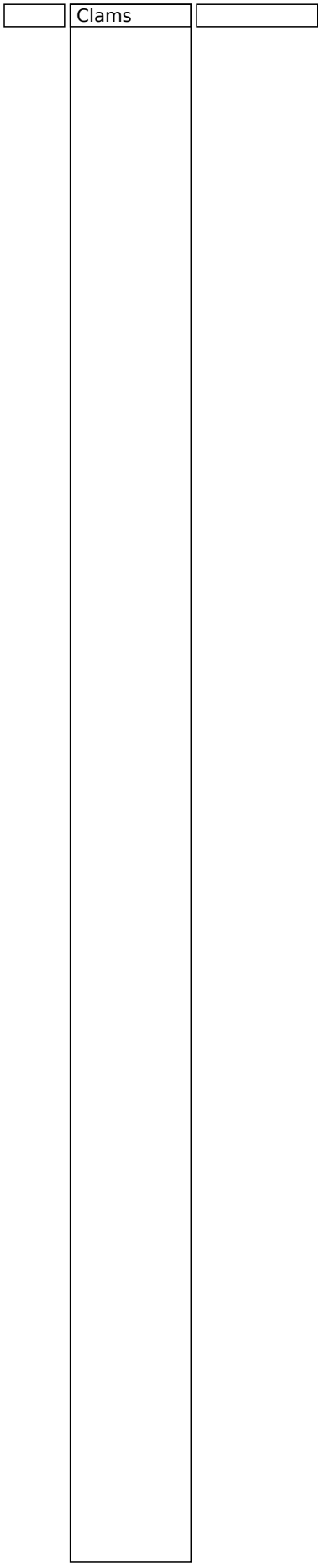
		Total genome, include microbial genom

5



	Muscle tissue	

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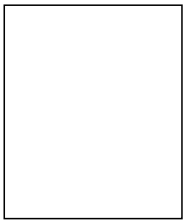
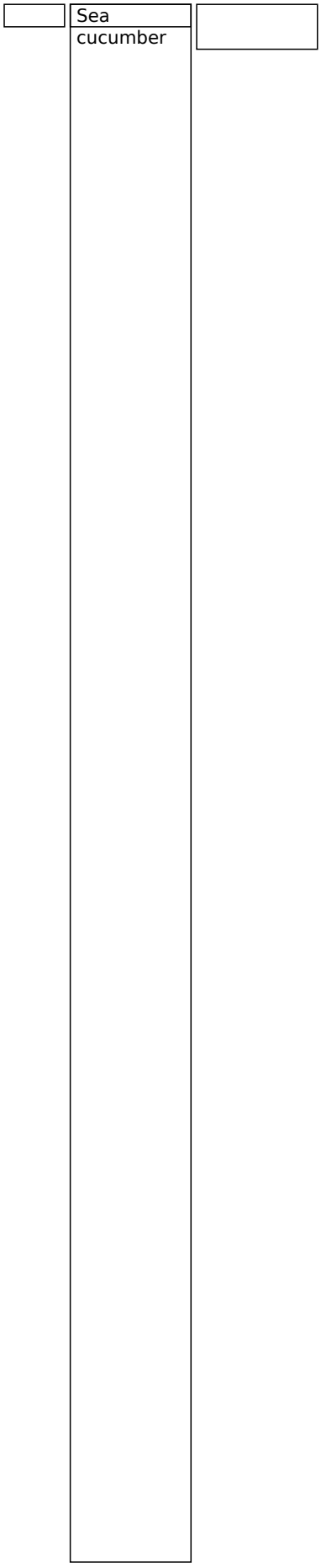
		~~Fragment of the mitochondrial COI gene~~ total genome

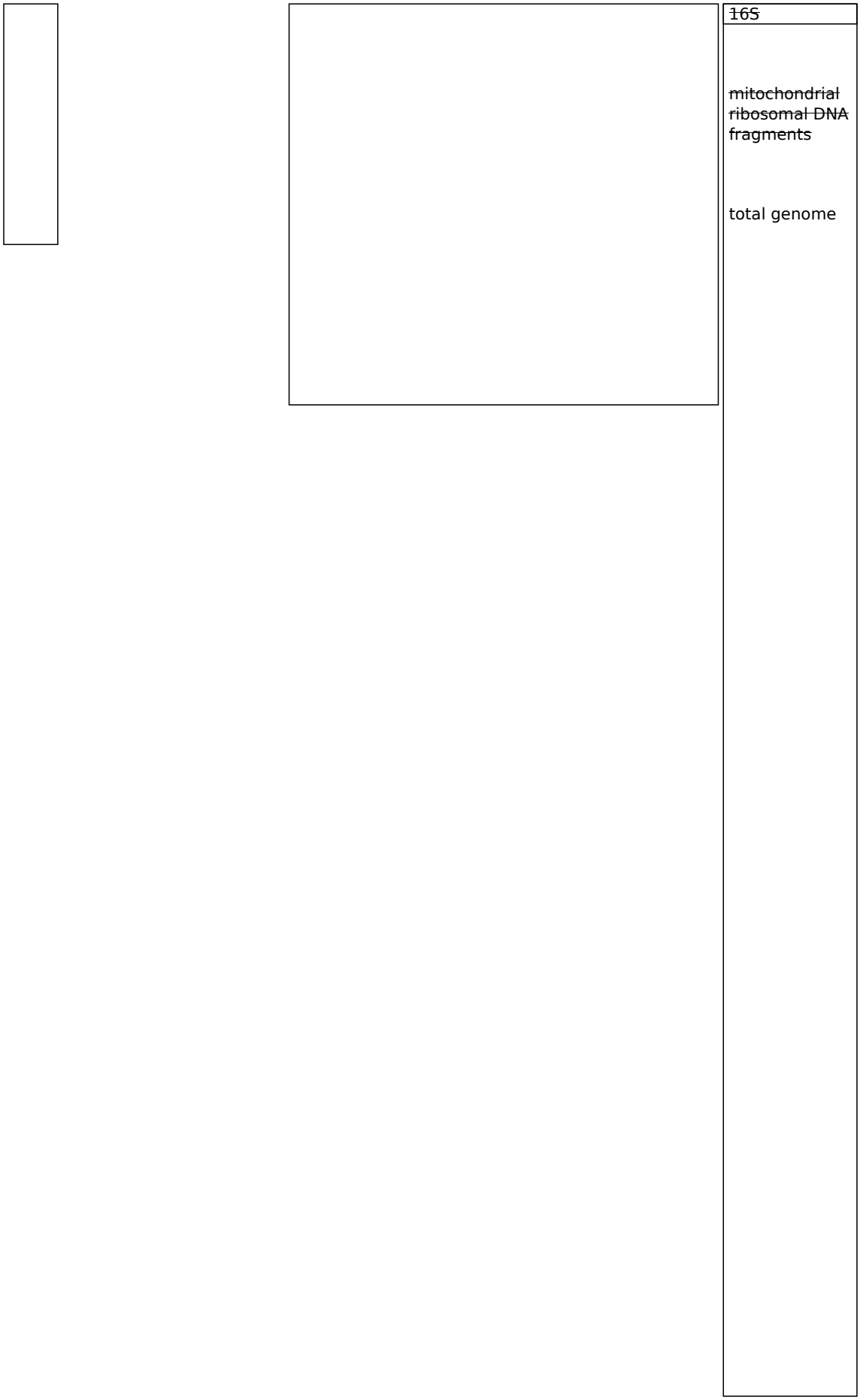
6



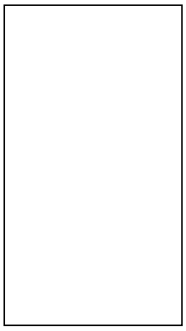


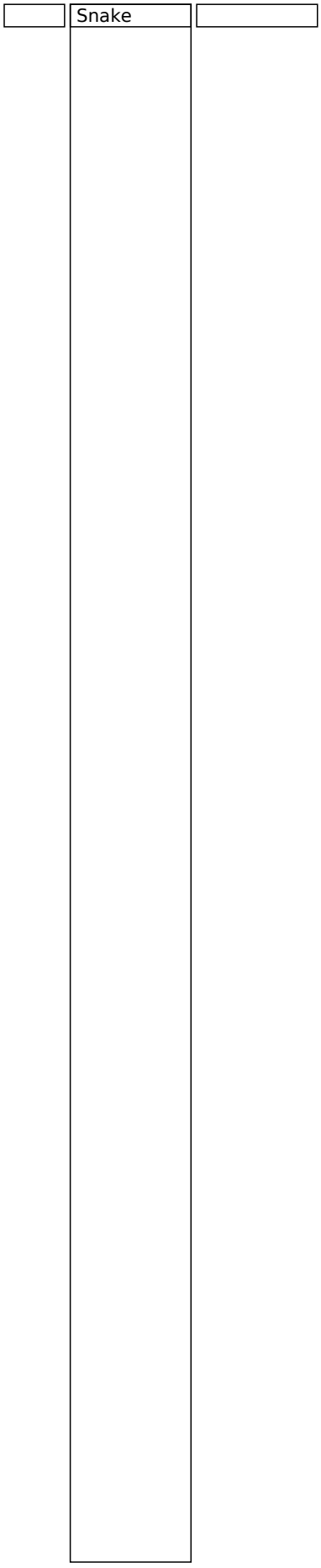
	Oral tentacle	

	Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice (-70°C). Typically 200 mg of oral tentacle was ground in liquid N2 in the presence of 600 µl of proteinase solution (50 mM Tris–HCl, pH 7.5, 50 mM EDTA, pH 8.0, 0.4% SDS, 0.5 mg/ml Proteinase K). The ground samples were immediately placed at 65°C for minimum of 2 h. The digested samples were repeteadly extracted with phenol:chloroform:isoamyl alcohol, 25:24:1. The resultant aqueous phase was adjusted to 0.5 M NaCl and to 1% cetyltrimethylammonium bromide (CTAB) and incubated for a further 20 min at 65°C. After two final phenol/chloroform extractions, total DNA was precipitated by adding an equal volume of isopropanol followed by sedimentation at 13,000 rpm for 20 min. The DNA pellet was rinsed with 500 µl of 70% ethanol, air-dried, and resuspended in 200 µl of TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA).	


~~16S~~
~~mitochondrial ribosomal DNA fragments~~
total genome

7





	Egg yolks, slough skin and non-invasively sampled (NIS)muscle, blood, and faecal material.	

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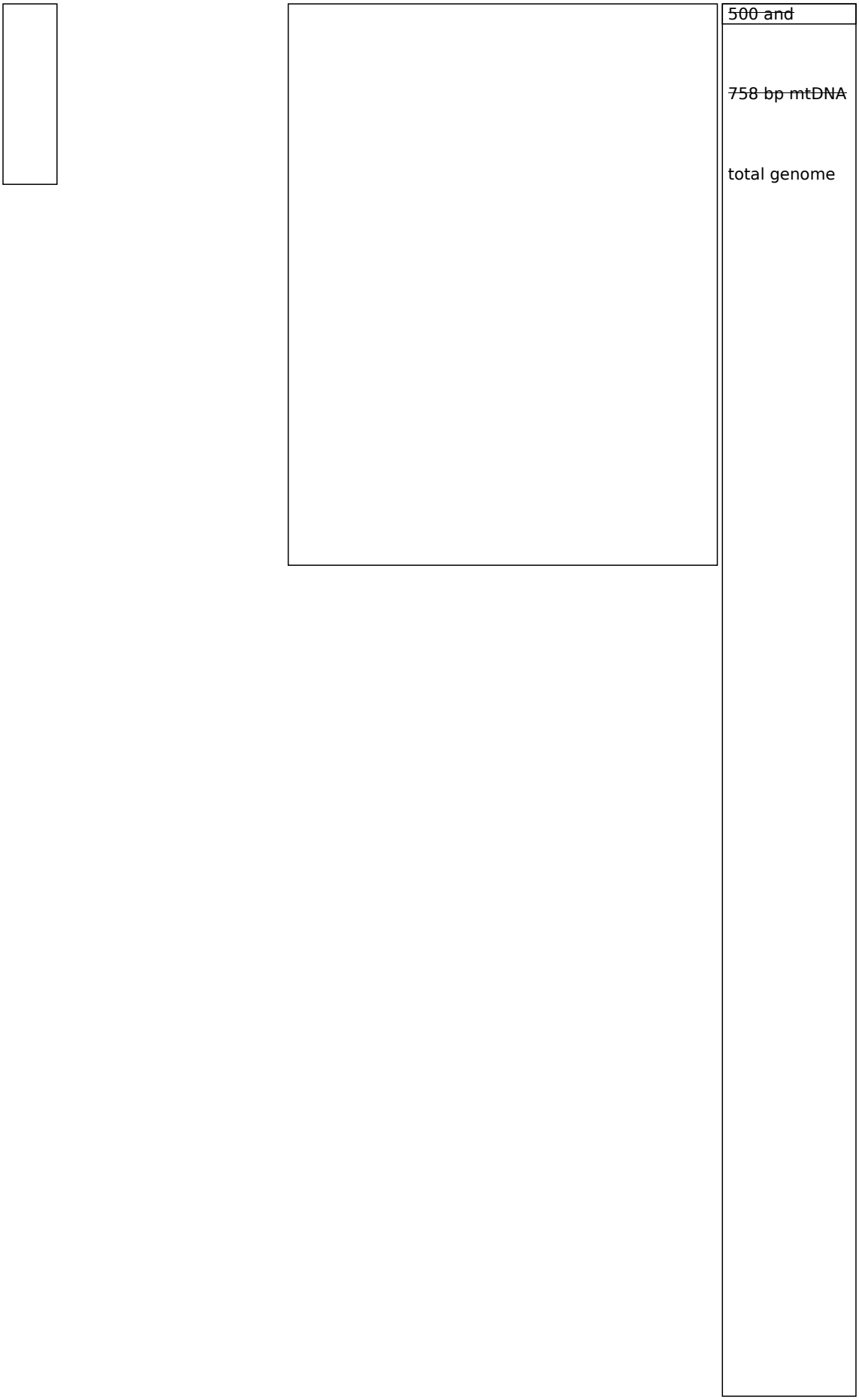
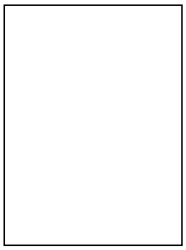
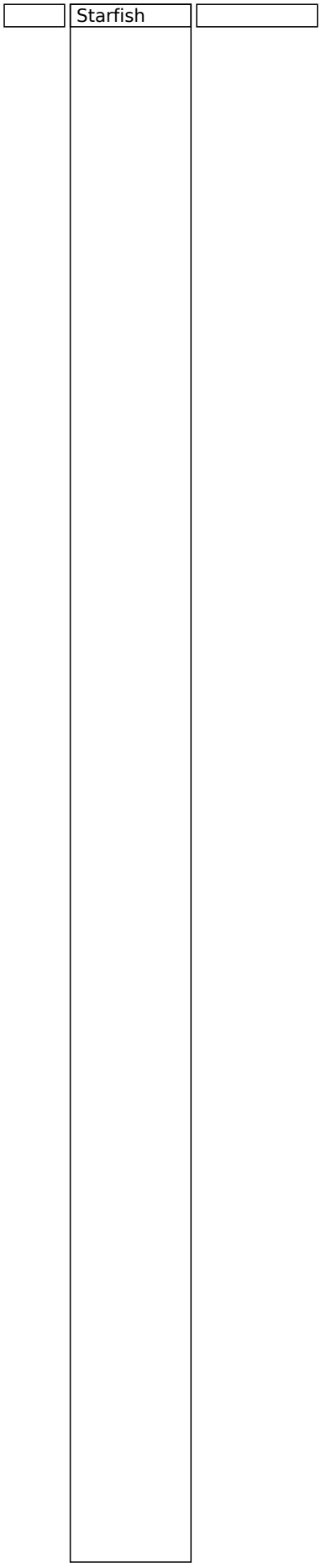

~~500 and~~
~~758 bp mtDNA~~
total genome

8





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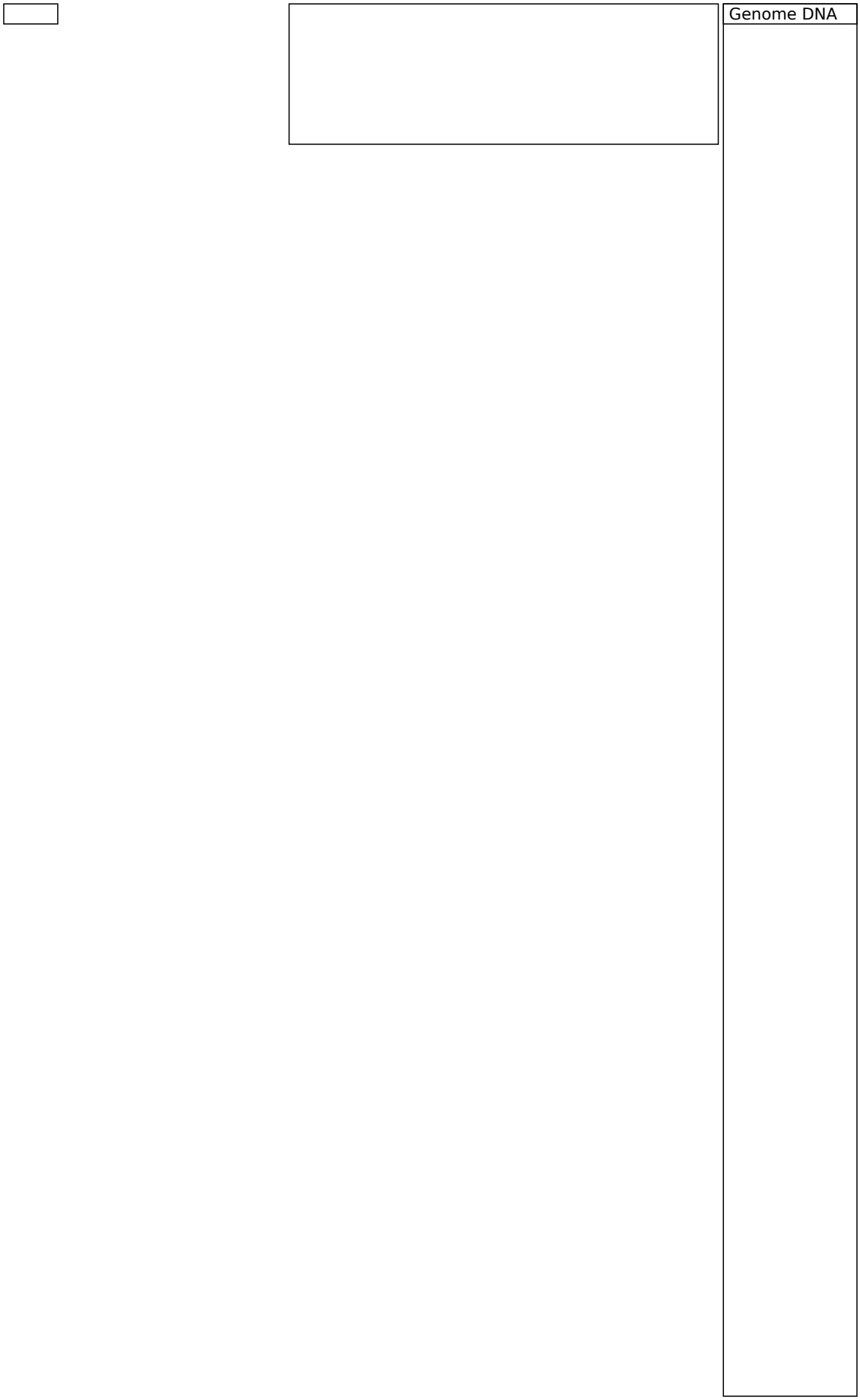

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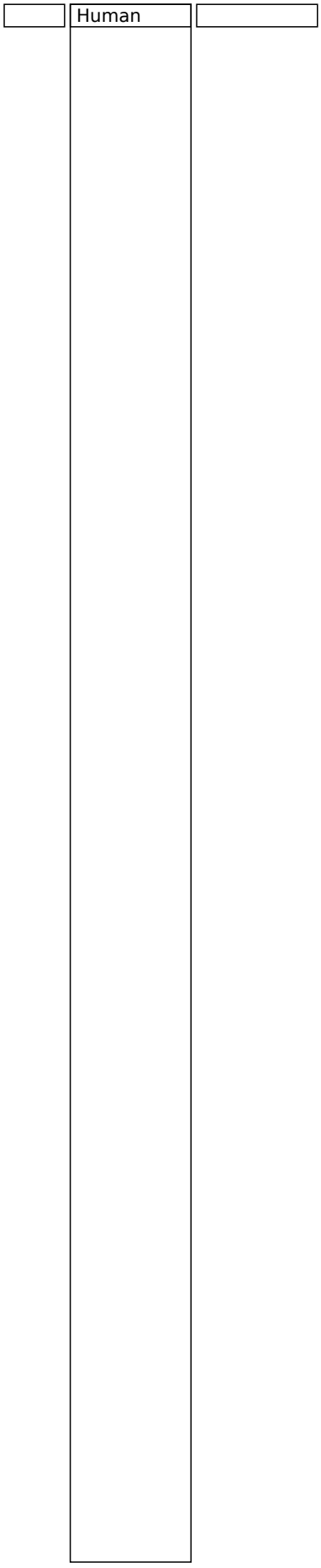

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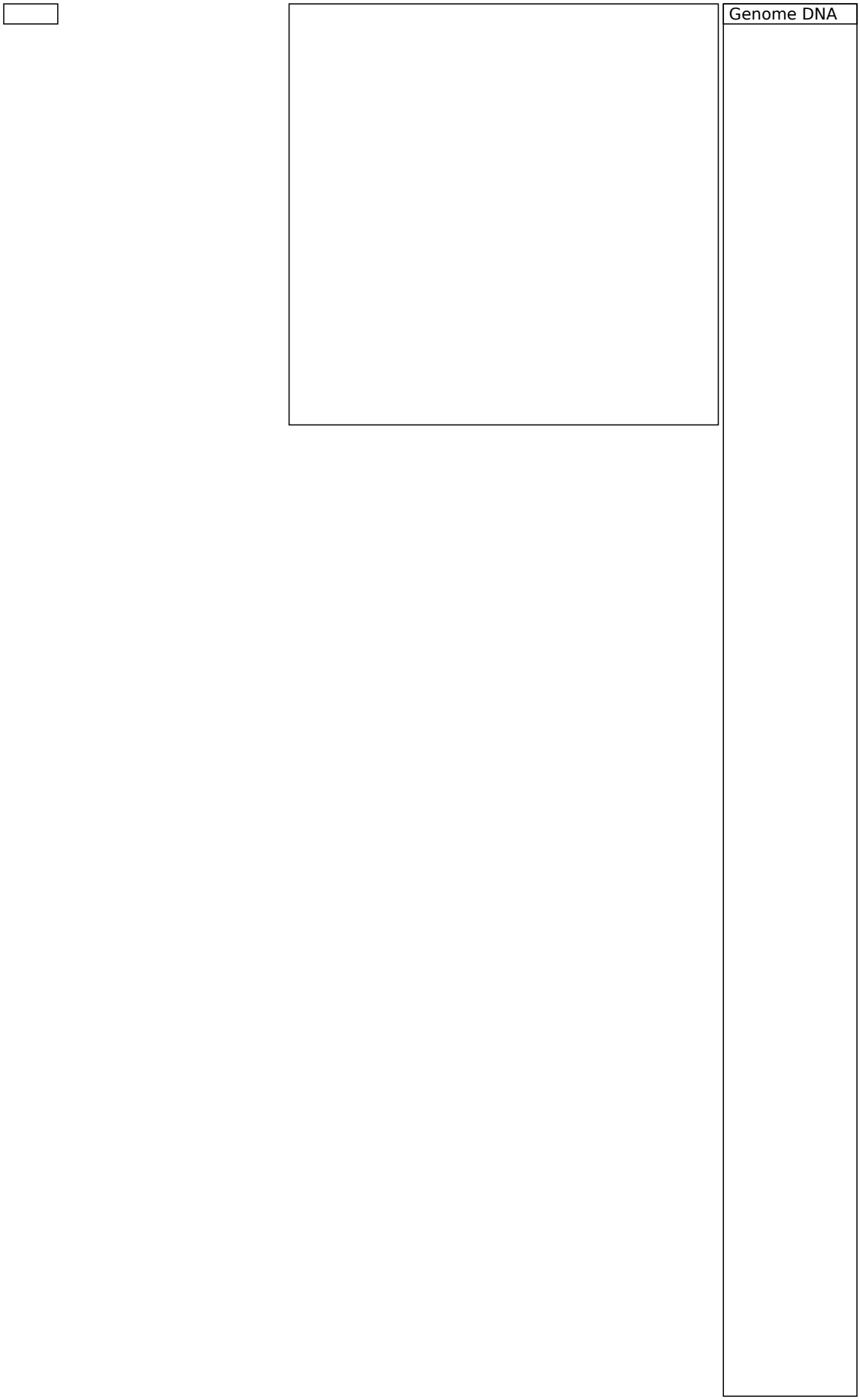

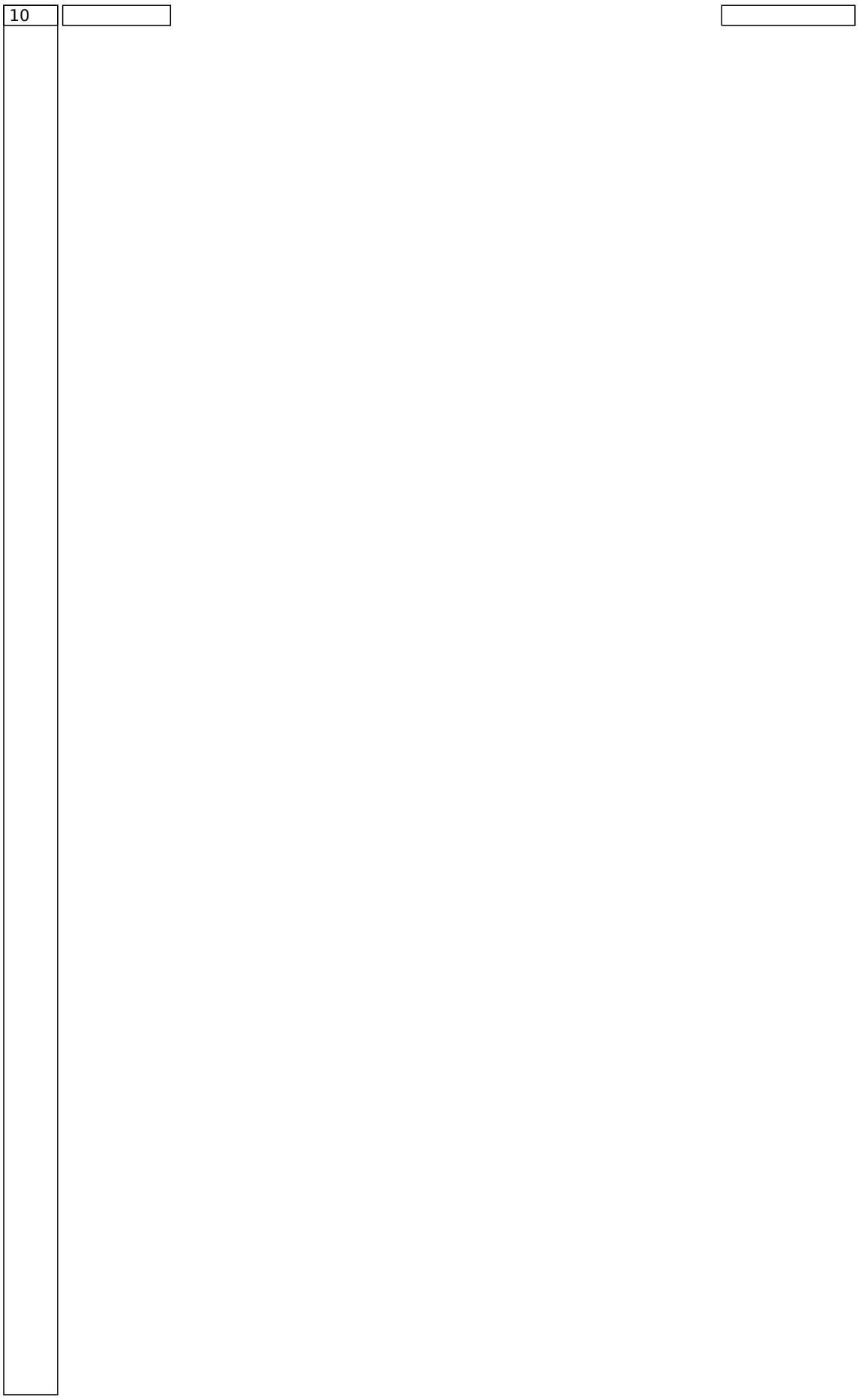

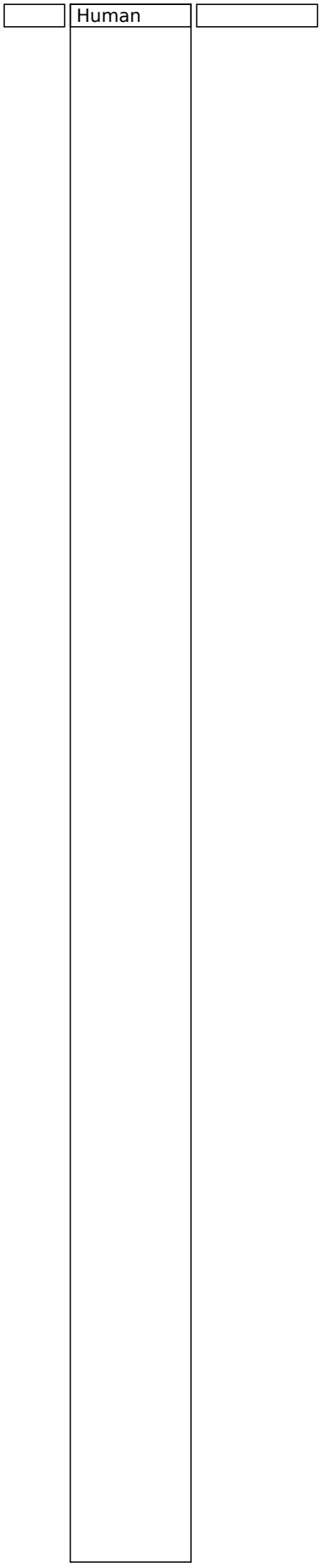

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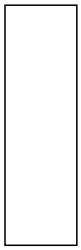
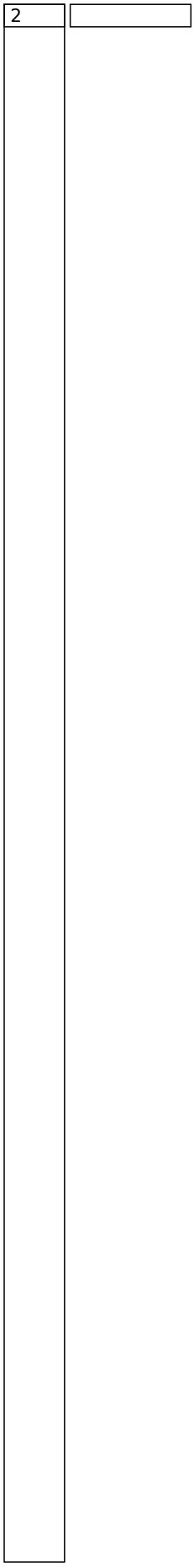
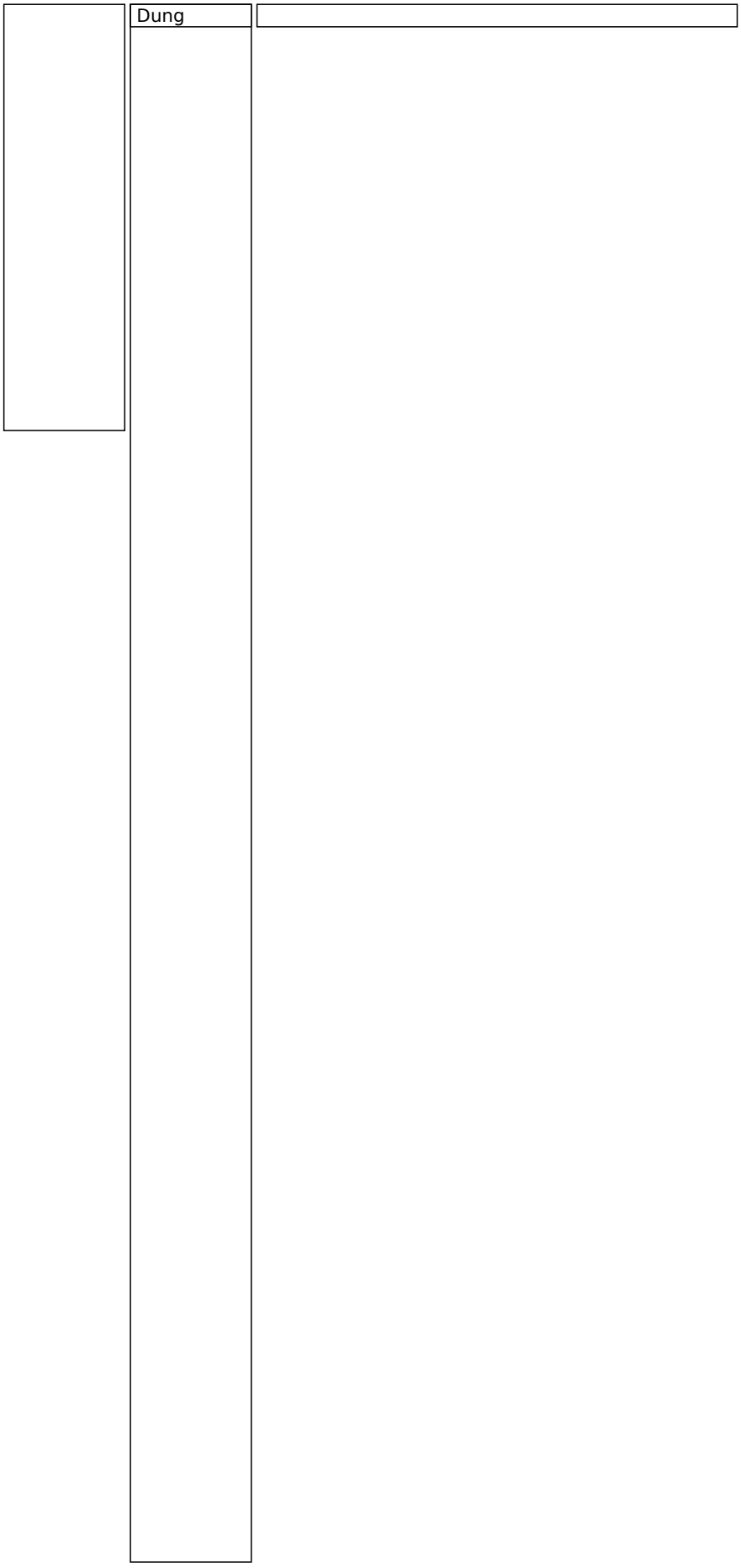
EKSTRAKSI DNA
Sri Bulan Musmiah G352110101

NO	SPECIES	SAMPLE	DNA EXTRACTION	GENOME TARGET
1	Moa(Aves: Dinornithiformes)	Bone powder	DNA was extracted from 200 mg of bone powder by incubation with rotation at 55°C for 48 h in 1.5 mL digestion buffer [20 mM Tris, pH 8.0, 1% Triton X-100, 10mM dithiotheitol (DTT), 1 mg/mL proteinase K and 0.47 M EDTA].The supernatant was spun through 30,000 MWCO Vivaspin columns and then combined with 5 volumes of PBI buffer (Qiagen, Valencia, CA) before the DNA was extracted using silica spin columns (Qiagen).	Mitochondrial control region Total genome

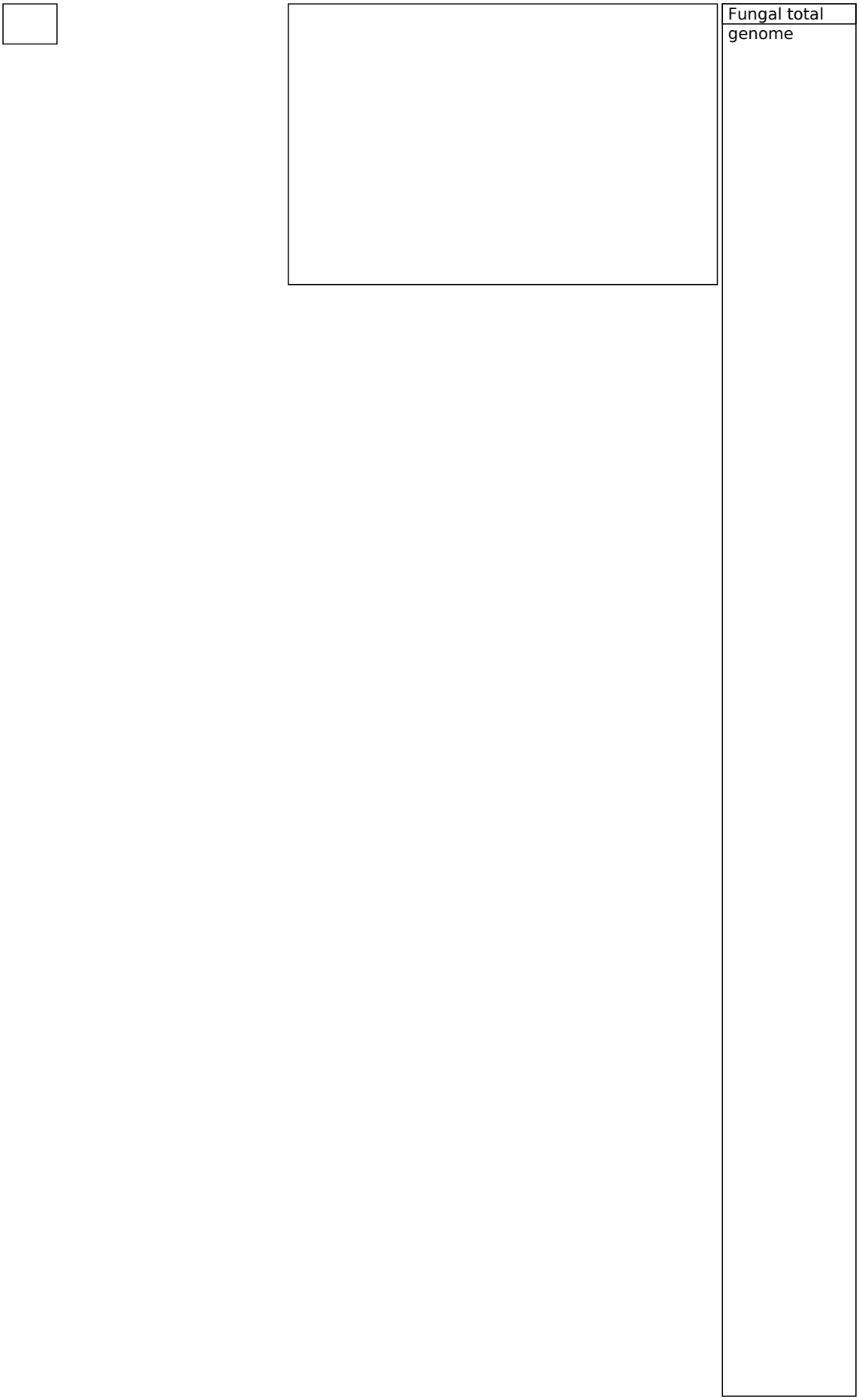


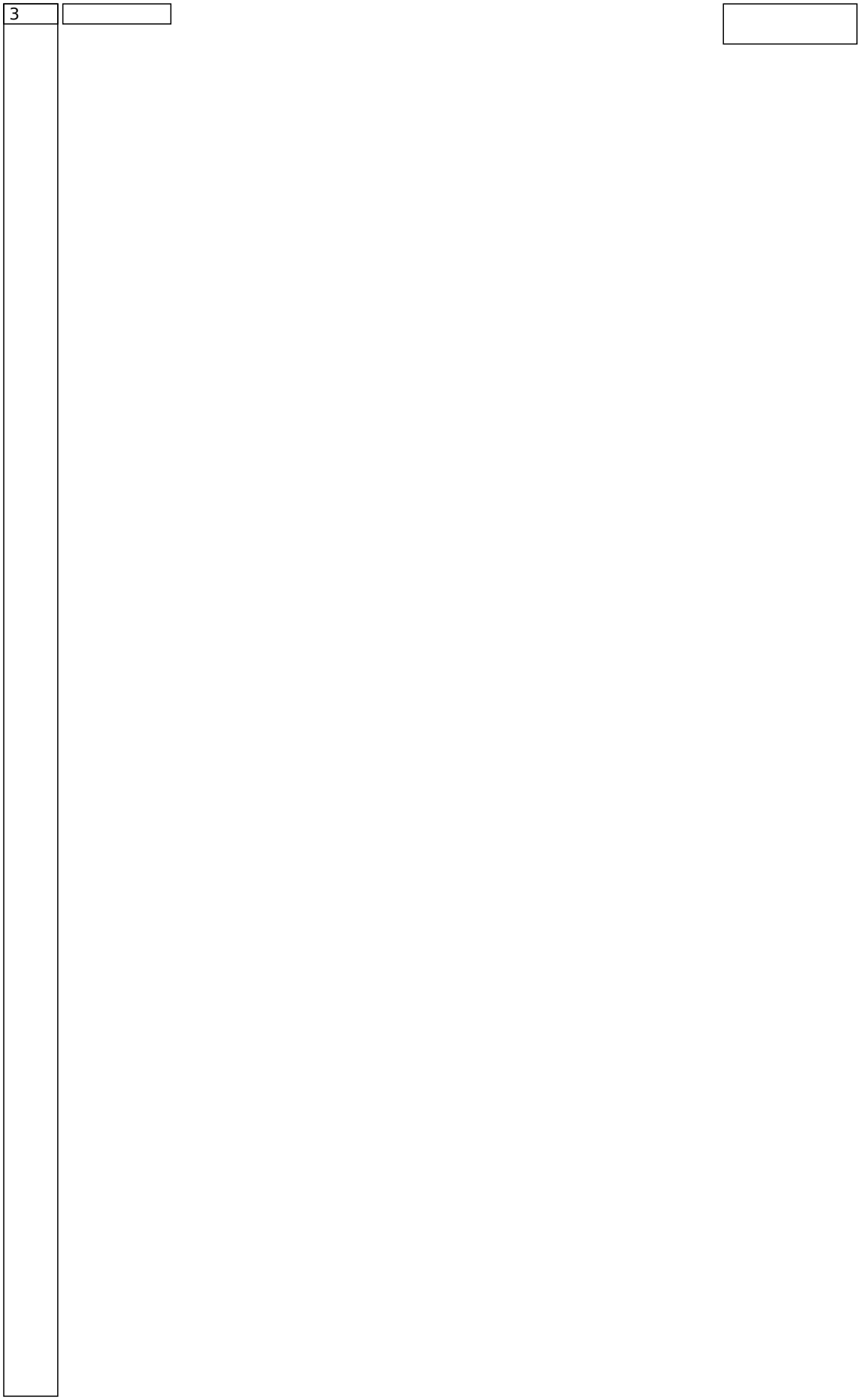

2

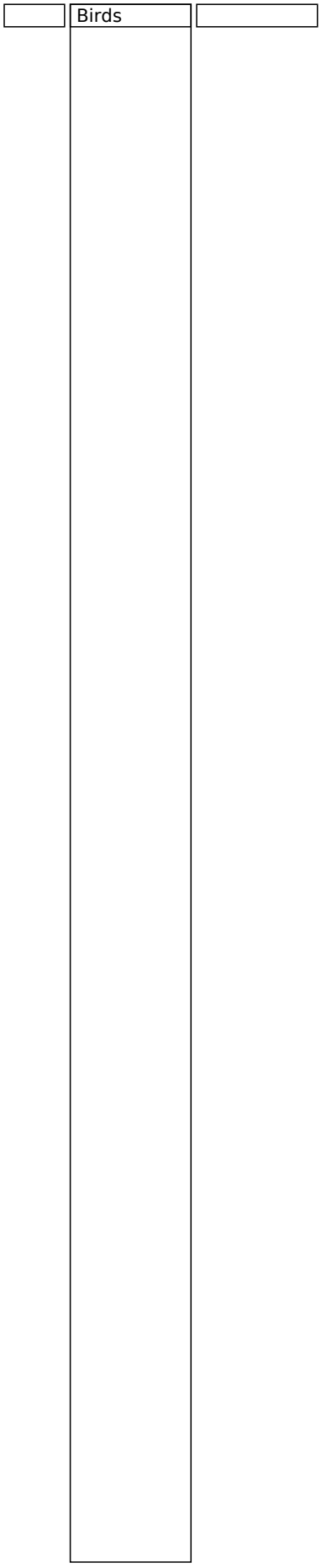
	Cows,prong horn, bison , black-tailed prairie dogs (Fungi?)	


Dung

	Fresh dung (pellets or portions of dung patties) was collected by observing focal individuals until they 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. DNA was extracted using the UltraClean® Fecal DNA isolation kit	


Fungal total
genome



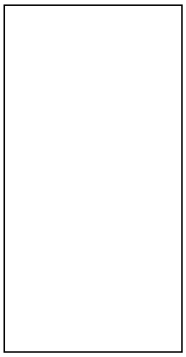


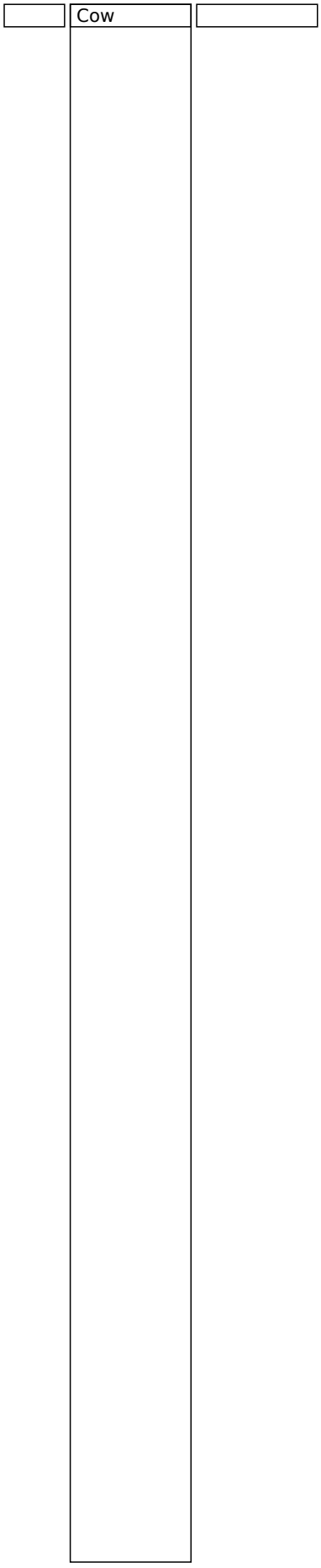
	Feather	

	DNA extraction of the archaeological feather followed the same protocols as the fresh samples. The protocols follows strict contamination-control for the analysis of ancient remains. Two and five feather barbs were removed from the feather shaft. Barbs were first rinsed in 3% sodium hypochlorite for 30 seconds to remove possible surface contamination, then 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 keratinis 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.	

		~~Fragments 200 to 300 bp in size of the mitochondrial DNA cytochrome b gene~~ Total genome - fragmented genome

4	



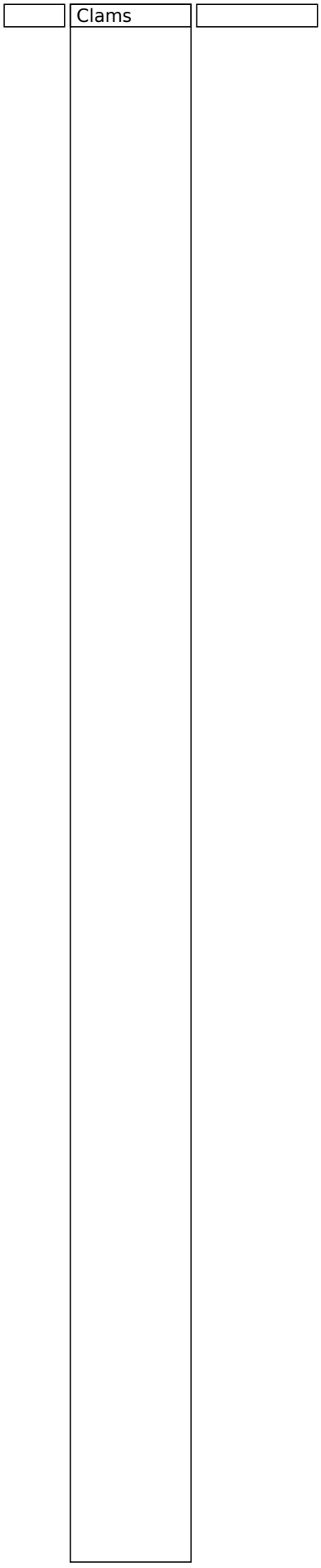


	Milk and curd	

	Total DNA was isolated from milk samples by a phenol/chloroform method, followed by ethanol precipitation according to Sambrook et al.(1989)Cheese samples were cut into small pieces, and total DNA from cheese matrix was obtained by performing phenol/chlorophorm as described above. DNA from reference strain of bacteria were prepared from pure culture according to the guanidium extraction procedure described by Pitcher et al. (1989).	

		Total genome, include microbial genom

5

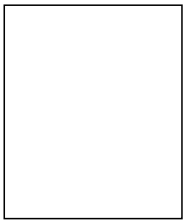


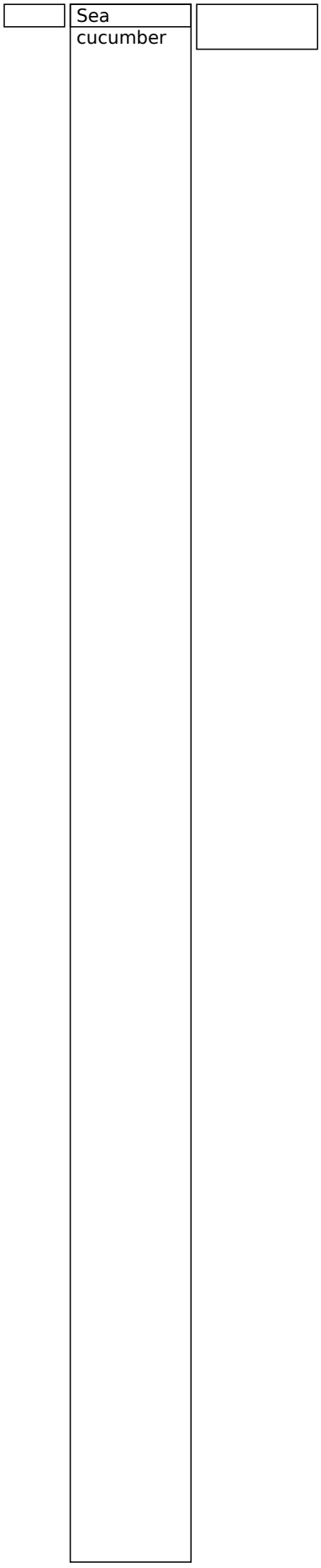
	Muscle tissue	

	Individuals with opened and closed shells were preservedfrom each beach in 80% ethanol. Muscle tissue was extracted from the middle and apex region (c. 1mm^2) of the foot and cleaned with ethanol (75%) to remove sand, detritusor external organic matter.DNA extraction was performed with the Qiagen DNAMini kit, with slighlty modification to increase the concentration of DNA, i.e. we used 150 µl of elution buffer.	

		~~Fragment of the mitochondrial COI gene~~ total genome

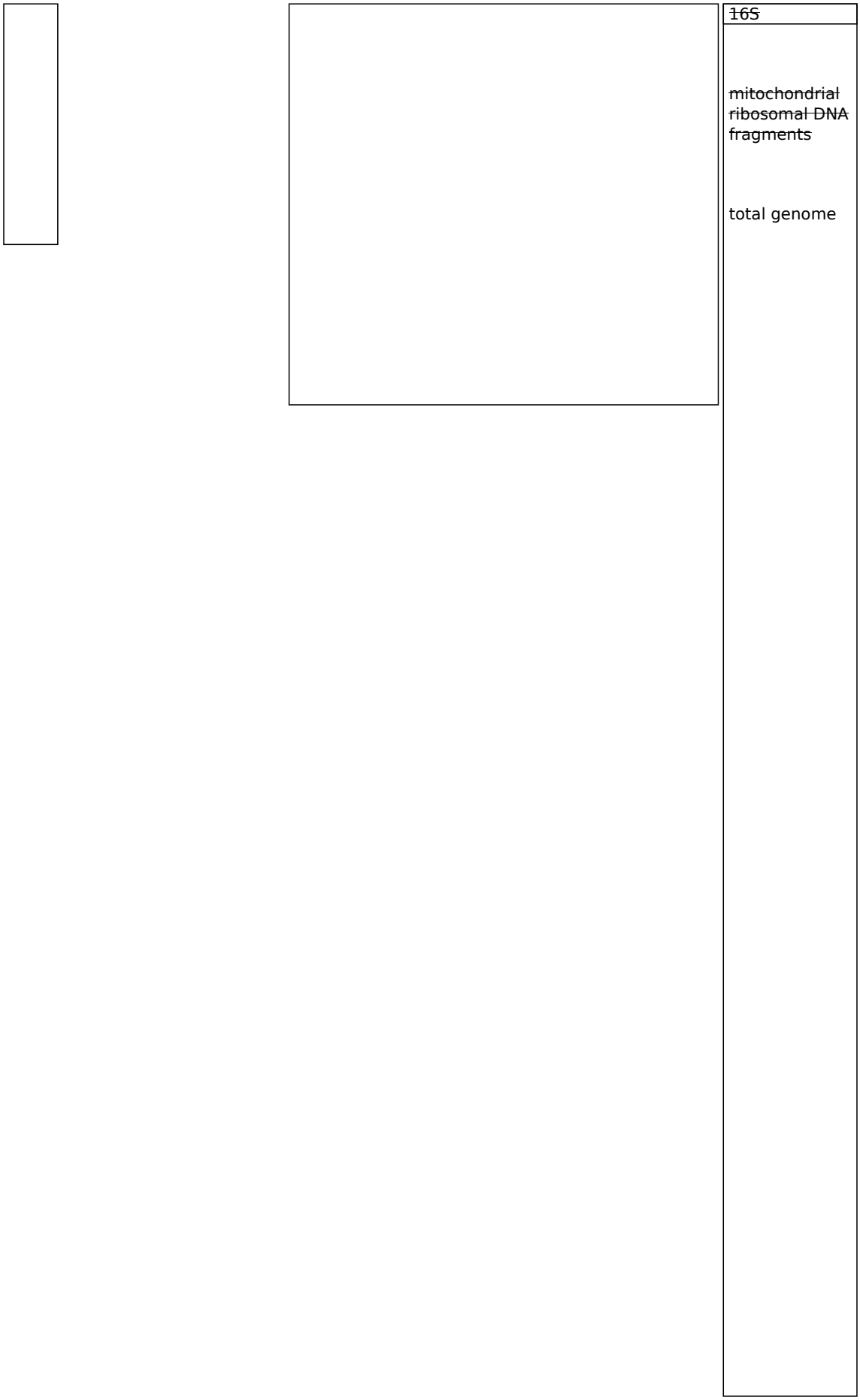
6



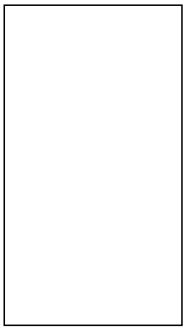


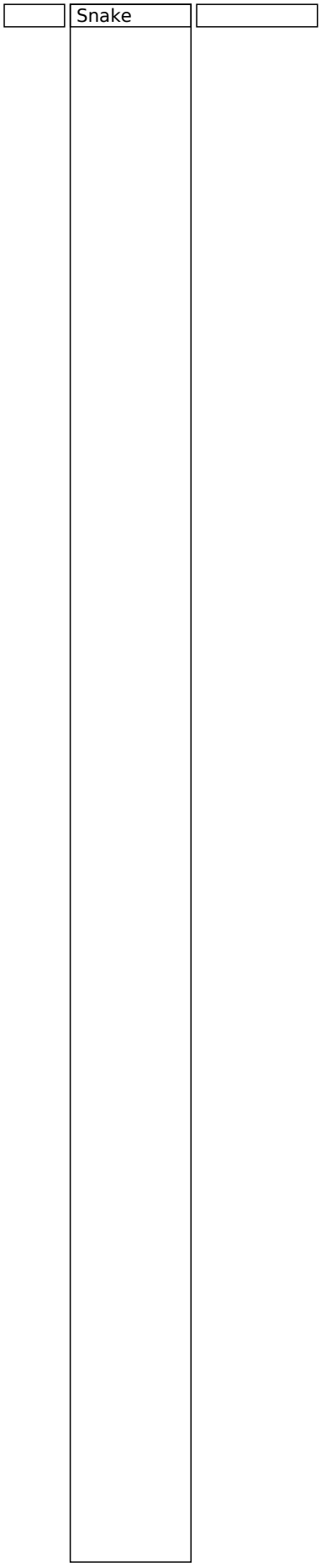
	Oral tentacle	

	Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice (-70°C). Typically 200 mg of oral tentacle was ground in liquid N2 in the presence of 600 µl of proteinase solution (50 mM Tris–HCl, pH 7.5, 50 mM EDTA, pH 8.0, 0.4% SDS, 0.5 mg/ml Proteinase K). The ground samples were immediately placed at 65°C for minimum of 2 h. The digested samples were repeteadly extracted with phenol:chloroform:isoamyl alcohol, 25:24:1. The resultant aqueous phase was adjusted to 0.5 M NaCl and to 1% cetyltrimethylammonium bromide (CTAB) and incubated for a further 20 min at 65°C. After two final phenol/chloroform extractions, total DNA was precipitated by adding an equal volume of isopropanol followed by sedimentation at 13,000 rpm for 20 min. The DNA pellet was rinsed with 500 µl of 70% ethanol, air-dried, and resuspended in 200 µl of TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA).	


~~16S~~
~~mitochondrial ribosomal DNA fragments~~
total genome

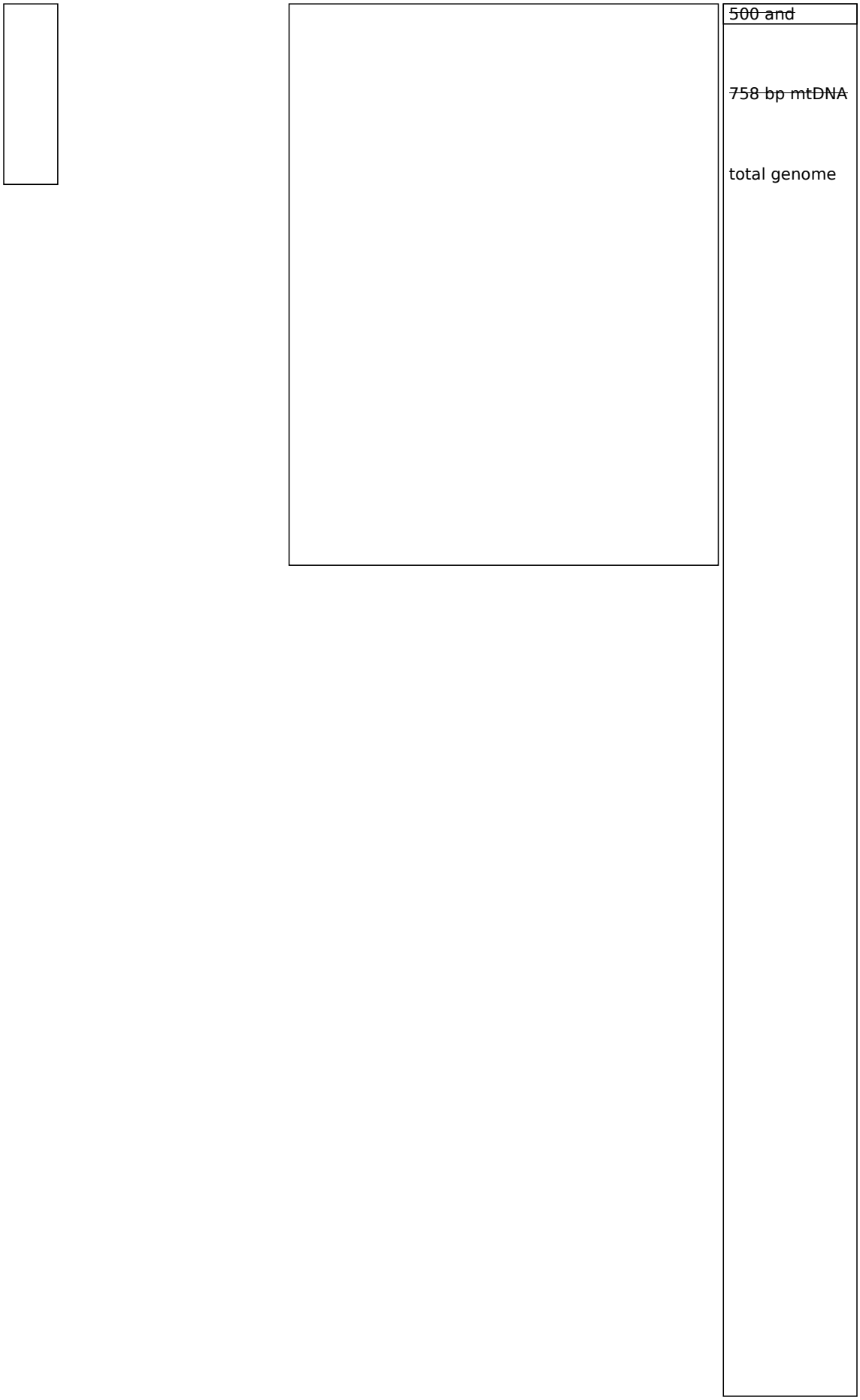
7



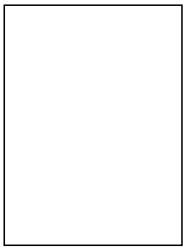


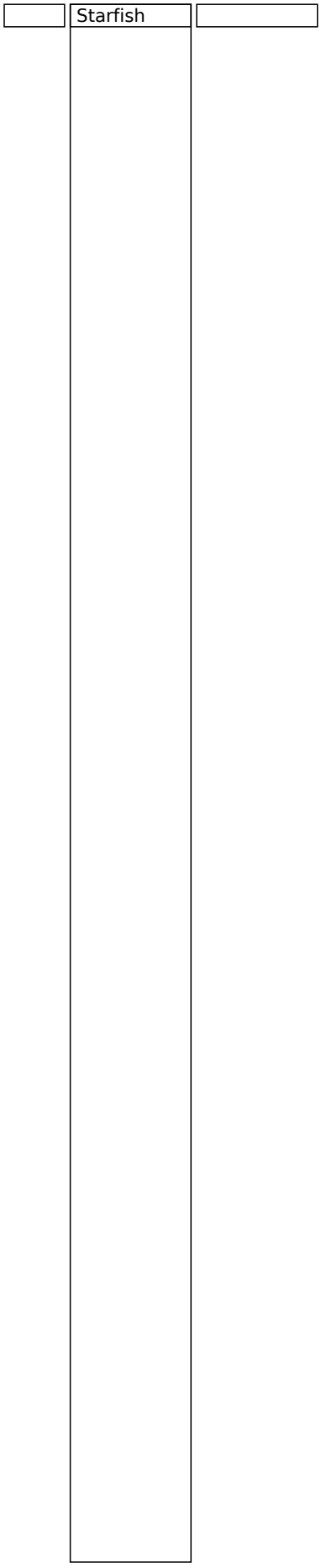
	Egg yolks, slough skin and non-invasively sampled (NIS)muscle, blood, and faecal material.	

	Field collected carcasses, road kills and foetal samples were directly frozen at -20'C or preserved in 95% ethanol. Ancient ethanol preserved tissue (A.E.P.T.; collected pre-1907 to 1969) consisted of museum samples. Non-viable grass snake eggs,located in compost heaps, were preserved at -20^{0}C. 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 -20ºC 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 cm^2) of slough was rinsed in 1 ml of ddwater at 55 ºC 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 cm^3), NIS muscle (1 cm^3) and blood (5 µl in Seutin'sbuffer) using QiagenDNeasy tissue extraction kit. Faecal material was extracted using QIAamp@ DNA stool mini kit.	


~~500 and~~
~~758 bp mtDNA~~
total genome

8





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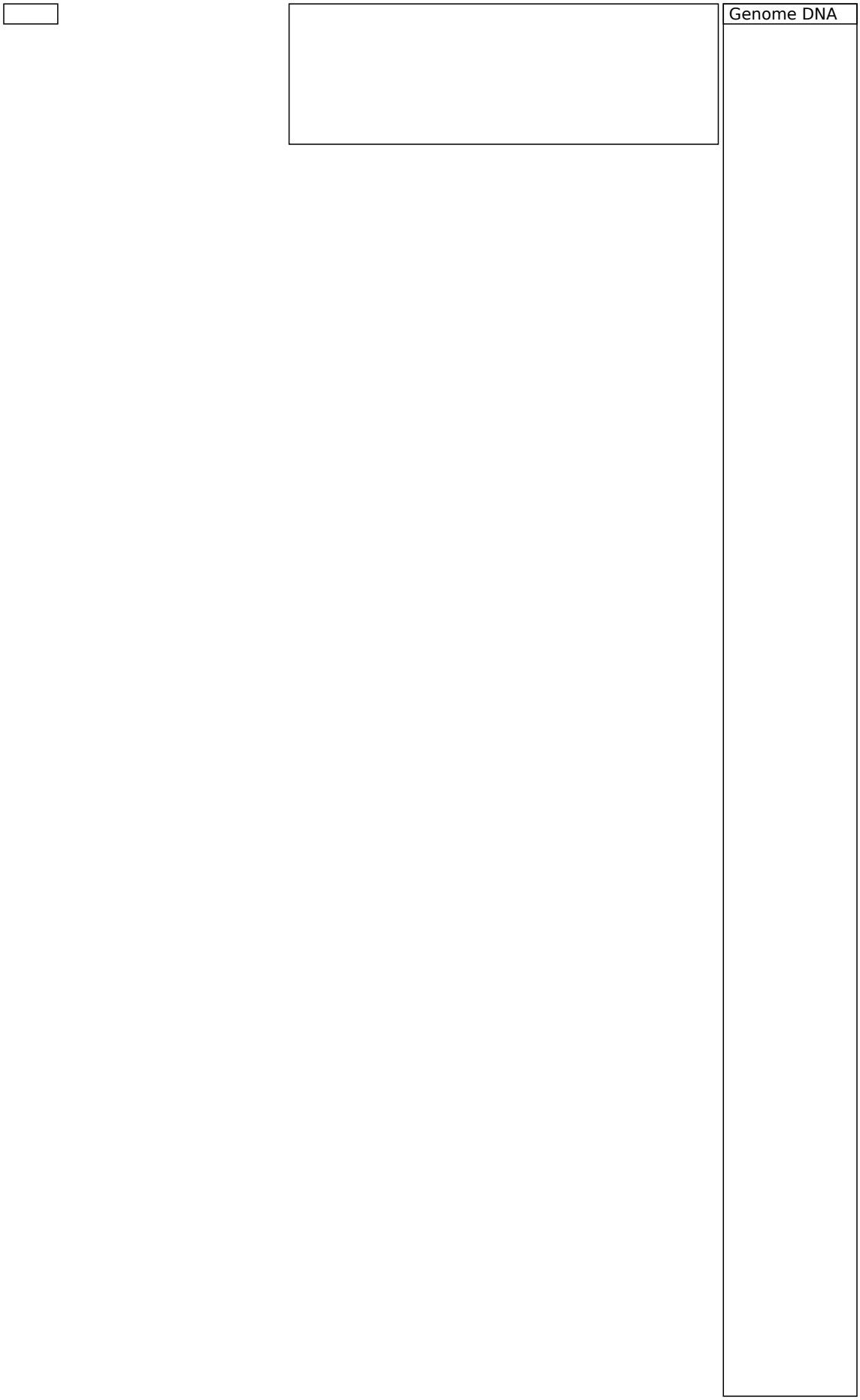

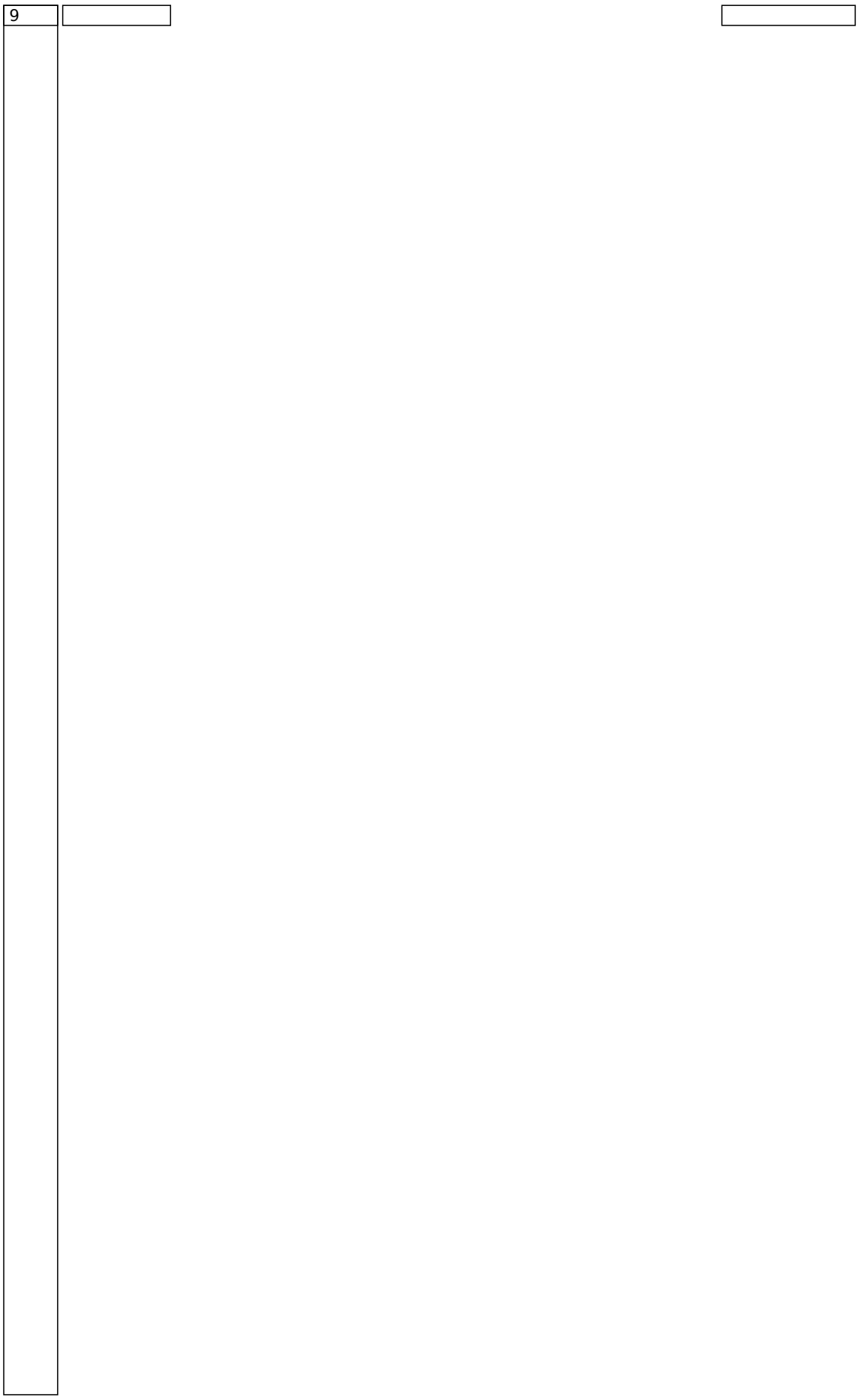

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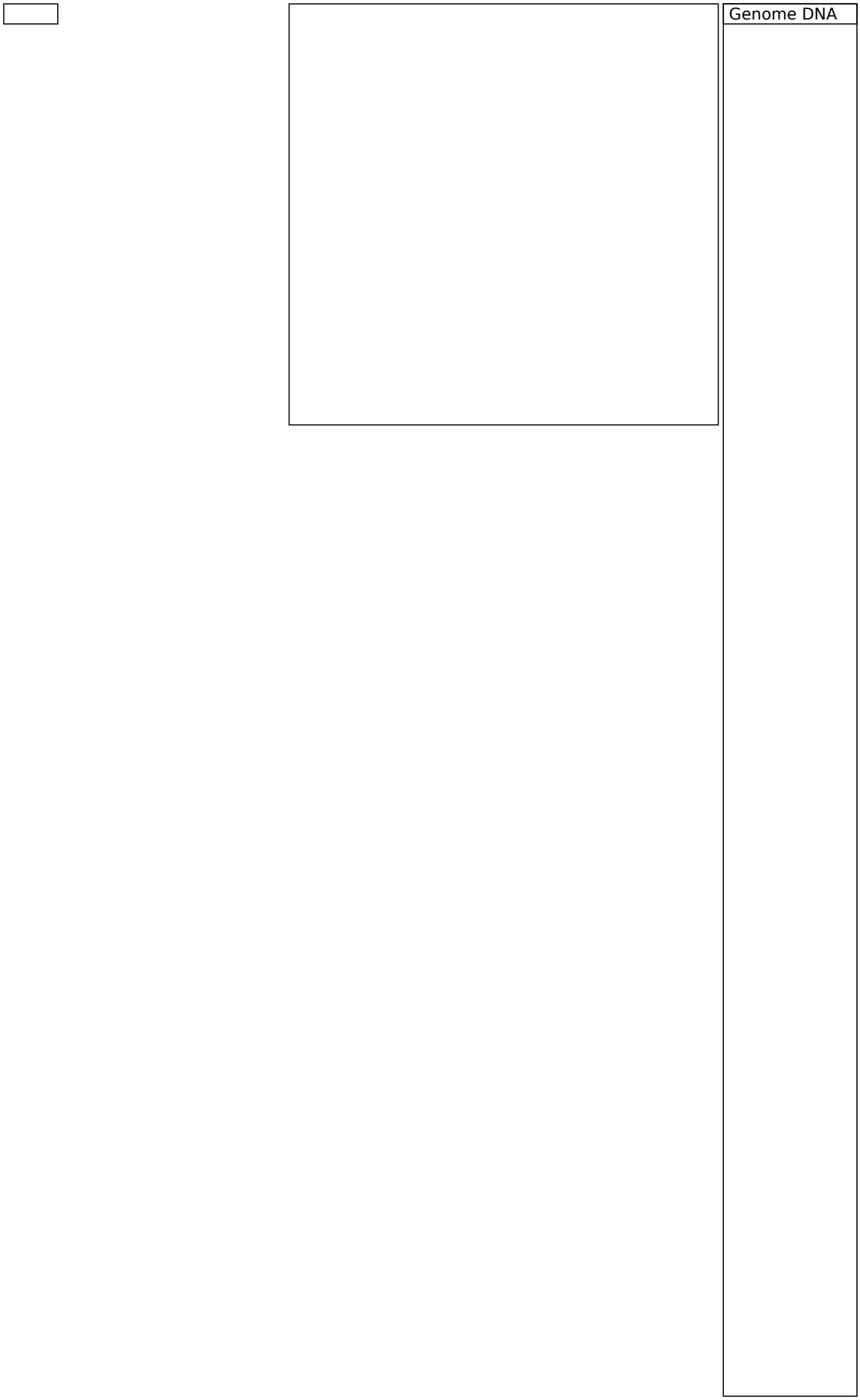

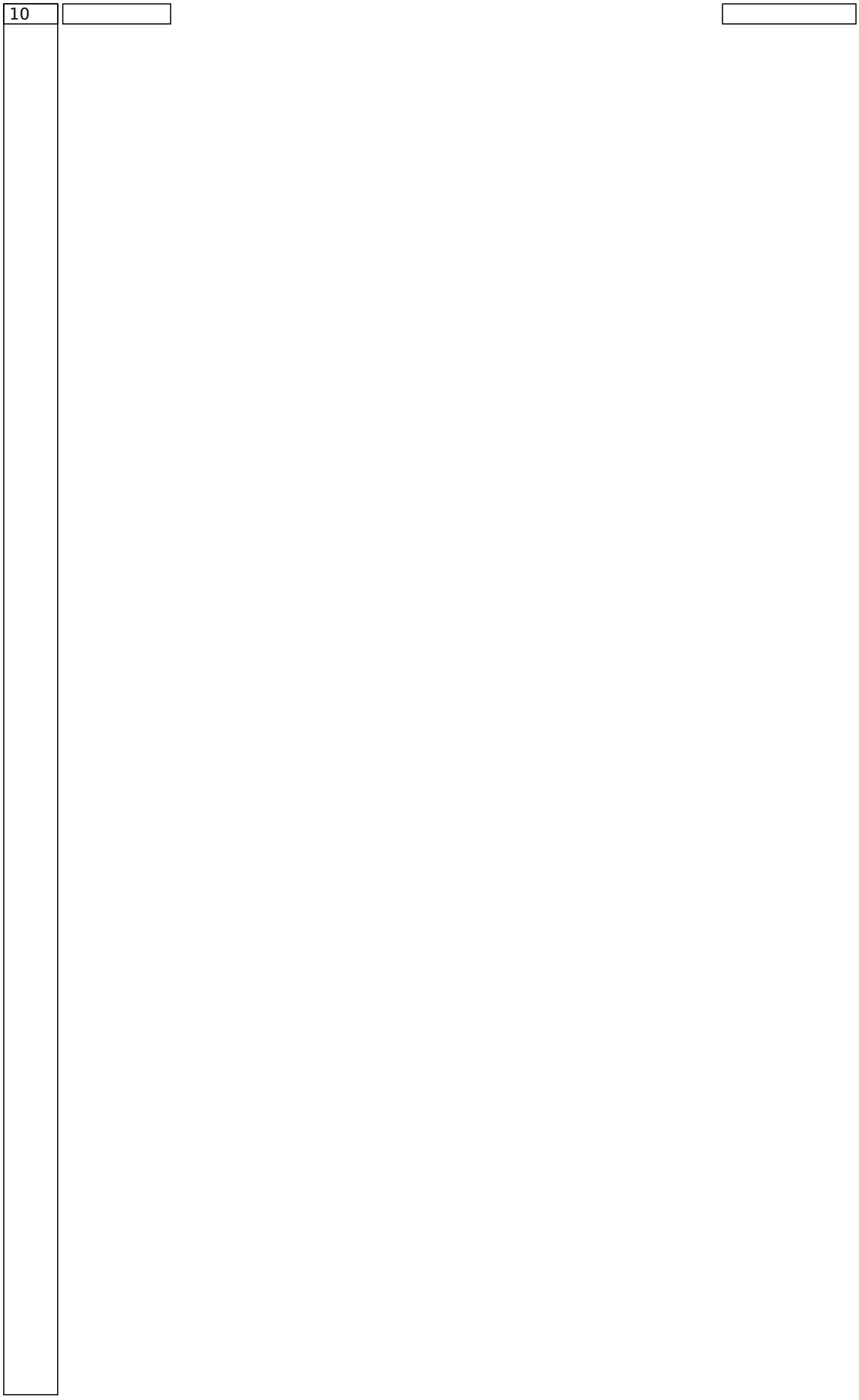

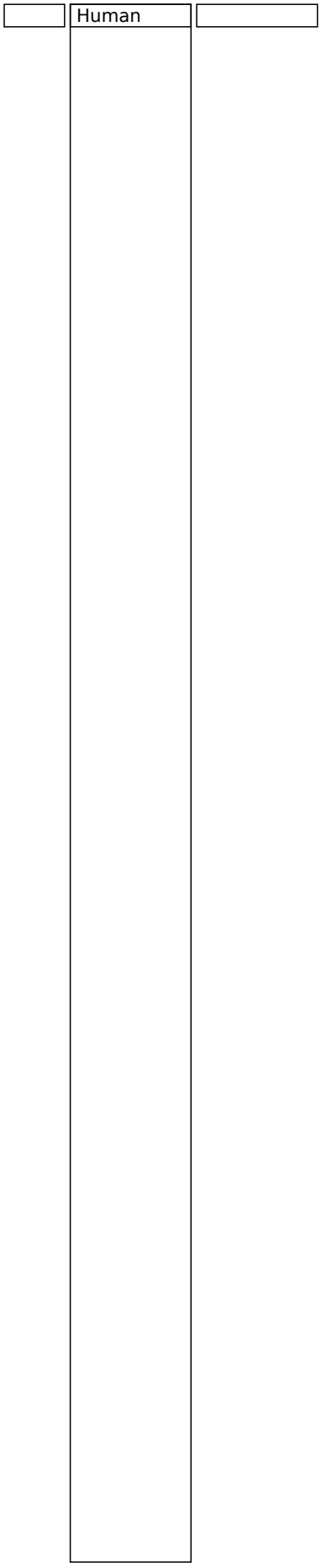

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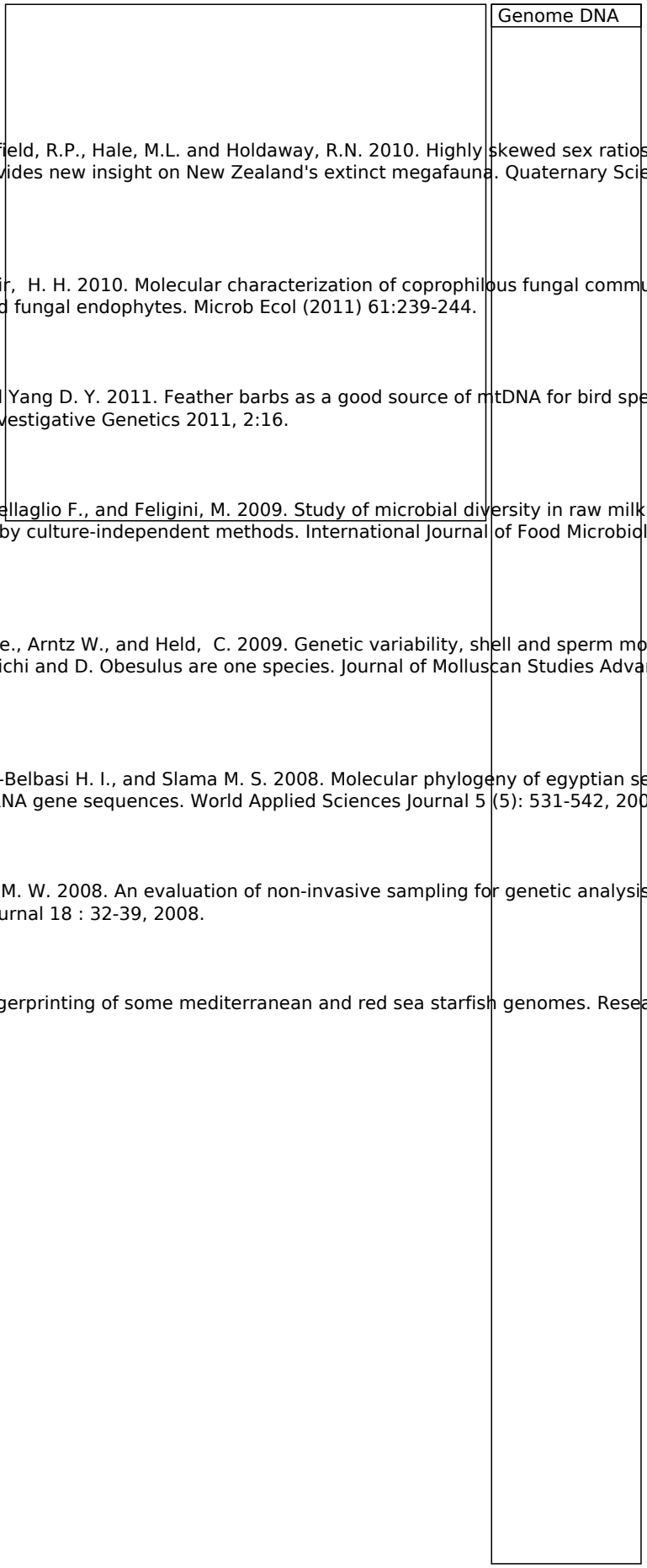

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