Supplementary material for:

A Rapid Protocol for Generating Arthropod DNA Barcodes Suitable for Use with Undergraduate Students

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Contents:

DNA Barcoding of spiders

Dr Grant R. Brown

Background and aims of the practical

DNA barcodes are short sequences of DNA that be used to assign an organism to a particular species. In animals, a 658bp long section of the mitochondrial CO1 (cytochrome oxidase subunit I) region is most often used for barcoding purposes. Mitochondrial DNA is useful for barcoding as it is very abundant in all eukaryotic cells, and has the property that it evolves rapidly, yet without extensive variation. Hence, these sequences are likely to be identical between all members of the same species, yet will be distinct from members of other species.

The purpose of this practical is to produce novel barcodes for spiders in the family Linyphiidae. 'Linyphiids' are small sheet-weaving spiders often known as "money spiders" in the UK. These spiders are common in almost every terrestrial habitat, and can be found throughout the year. In common with most arthropods they have been generally neglected in research and only a small proportion of the European fauna has been barcoded (around 30%). You will therefore have the opportunity to contribute data new to science during this practical, by generating morphological and DNA vouchers for an important yet understudied group of animals. You will extract the DNA using tissue from the legs of the specimens, and prepare the samples using forward and reverse primers for Sanger sequencing at Edinburgh. The sequences generated during the practical, once returned to us, will be used in the assessment.

Assessment

This practical is assessed, and to some extent the success of your assignment depends on obtaining good sequences. **It is vital that you pay close attention to the method below when preparing your specimen, and that you observe good clean working practice to avoid contamination.**

Full details of the assessment can be found at the end of this protocol. But in summary:

1. *You will assemble FASTA sequences for your two species using the chromatograms returned from the Edinburgh sequencing facility* **(80%)**.
2. *You will be assessed on your ability to interpret and synthesise the molecular and morphological sources of evidence you obtained* ***(20%).***

Overview and procedure

The practical is split into 2 sessions. In the first session you will prepare samples for DNA extraction, and in the second you will check the extractions were successful before preparing them to be sent away for sequencing. You should have plenty of time to complete the practical, but it will require consistent attention as mistakes can easily creep in. If you keep good notes and observe good clean working practices however, you should be able to generate good PCR products for sequencing.

DAY 1 – SPECIMEN IDENTIFICATION AND DNA EXTRACTION

Read this protocol in full before commencing any lab work.

You will be given 2 species to work on. The purpose of this first session is to familiarise yourselves with the external anatomy of spiders and prepare your samples for DNA extraction. By the end of this session you will have attempted to identify your specimens to species on the basis of their external morphology (using the provided keys).

Throughout the practical work you should observe good laboratory practice, ensuring that you wear gloves when performing any of the DNA handling steps.

Procedure

1. At some point prior to preparing your DNA extraction samples, you should practice pipetting a known volume of water into a sample tube; check your accuracy using a balance (assume 1µl H_2O = 1µg). Record your accuracy in the table below for 3 trials using volumes below. If your accuracy is less than 98% speak to the demonstrators who will suggest improvements to your technique. **A major source of failure in DNA extraction arises from poor pipetting technique.**

Quantity	Weight (attempt 1)	Weight (attempt 2)	Weight (attempt 3)	Mean accuracy (%)
100µl				
10µl				

2. The spider specimens have been stored in 70-100% ethanol to preserve them and maximise the chances of obtaining useable DNA. **Work on one specimen at a time**, so that the risk of cross-contamination is lessened.

3. **Note down the original specimen label details accurately and in full in your lab notebook. Sequences cannot be published without these details and you will be asked to report them in full as part of the assessment**. Specimen labels will have some or all of the following information:

   Species name, sex, abundance,
   Collection locality (e.g. Bankhead Moss NNR, Fife)
   Locality grid reference (using the British National Grid – e.g. NO1400321)
   Collection date, Collection method (e.g. Pitfall trap), the name of the collector,
   Storage fluid (e.g. 70% etOH), Museum accession number (e.g. G7512.1828)

4. Place the specimen into a small inkwell containing some glass beads and carefully flood this with 75% ethanol. **You must keep the specimen immersed during inspection, otherwise the DNA and morphological structures will degrade and render the specimen useless**.

5. With reference to *Appendix i*, briefly inspect your specimens and make sure you can identify the major morphological structures (in particular, the palps/epigyne and the segments of the legs).

6. Accurately measure the body length (from the anterior of the cephalothorax to posterior of the abdomen) of each specimen using the eyepiece graticule and record this for your notes. The size of graticule divisions is given below:

Magnification	Size of graticule division (mm)
6.3x	0.15
10x	0.1
16x	0.06
25x	0.03
40x	0.02

7. Next, suitably orient the specimen and determine:
   a) The tibial spine formula (how many tibial spines are on each leg, from leg I to leg IV. Record your answer as 2-2-2-2, 1-1-0-0, etc.)
   b) The leg I metatarsal trichobothrium ratio (see figure below, and show your full working)
   c) The presence/absence of a leg IV trichobothrium (figure 1).

   We will need these three trait measures to facilitate identification to species level.

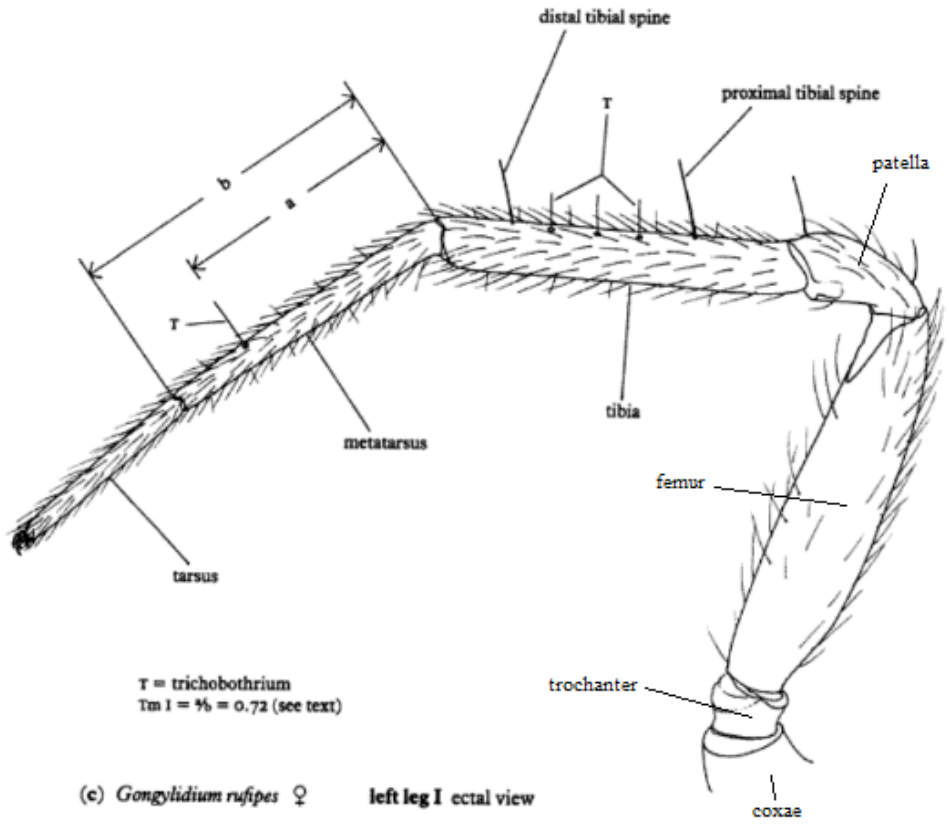


Figure 1. Diagram of leg I with segments named and the positions of trichobothria (T) and tibial spines indicated. The leg I metatarsal trichobothria ratio is the fraction a/b; i.e. the distance from tibia/metatarsus junction to the trichobothrium (a), over total length of metatarsus (b). Figure modified from Roberts (1987).

8. Identify your specimen to the lowest possible taxonomic resolution using the provided keys and help from the demonstrators. **Draw the specimen from both ventral and dorsal aspects**. **Special attention should be paid to illustrating the relevant genitalia. Male palps should be drawn from an ectal view, and the female epigyne should be drawn from the ventral aspect**.

9. If your specimen is <5mm in length, remove all of the legs at the coxa-trochanter joint using fine forceps. If your specimen is >5mm in body length, then only remove the legs from one side of the specimen.

10. Dab away any excess ethanol from the legs and place them into a clean autoclaved 1.5ml Eppendorf tube. Add 100µl of the working stock (fly squishing buffer + 20mg/ml Proteinase K) to each sample. **Prepare each species in this manner and label the tube so they can be uniquely identified**. Place the samples in the incubator which is set at 55^{o}C. We will leave them overnight to allow time for DNA extraction.

    The specimens will checked after the practical by GRB: it is essential that your extraction samples and the original specimens can be consolidated.

DAY 2 – DNA AMPLIFICATION AND CLEANING

The purpose of this session is to check that your extractions were successful and to then prepare these extractions for sequencing at an external facility. You will first run the DNA extract samples through a PCR cycle to amplify any digested DNA. Next, you will confirm the extracted DNA is of the correct length (~650bp) using an agarose gel and DNA. You will then 'clean up' the PCR product using Exo-star 1-step, and quantify the final DNA concentration using a second gel. Lastly, you will label and pack separate forward and reverse samples for each of the species you prepared, for shipping to the sequencing facility.

Ultimately, you will produce two samples for each species using the forward (LCO1490) and reverse (Chelicerate Reverse 2) primers.

Procedure

1. Retrieve your leg extractions from the incubator. Place them in the heat block @ 95°C for 2 minutes to halt the action of Proteinase K. You will now prepare the extractions for the initial PCR step.

You will need one 0.2ml Eppendorf for each species, plus another for the negative control. Remember to label your tubes! The negative control is this mixture without any DNA sample (use an extra 1µl of water instead). Each sample tube should contain the following:

Each sample (species) requires:	0.4µl of LCO1490 (@50µM/ml) (forward primer)
	0.4µl of Chelicerate Reverse 2 (@50µM/ml) (reverse primer)
	1µl of the DNA extraction sample
	8.2µl clean H_2O
	10µl myTAQ

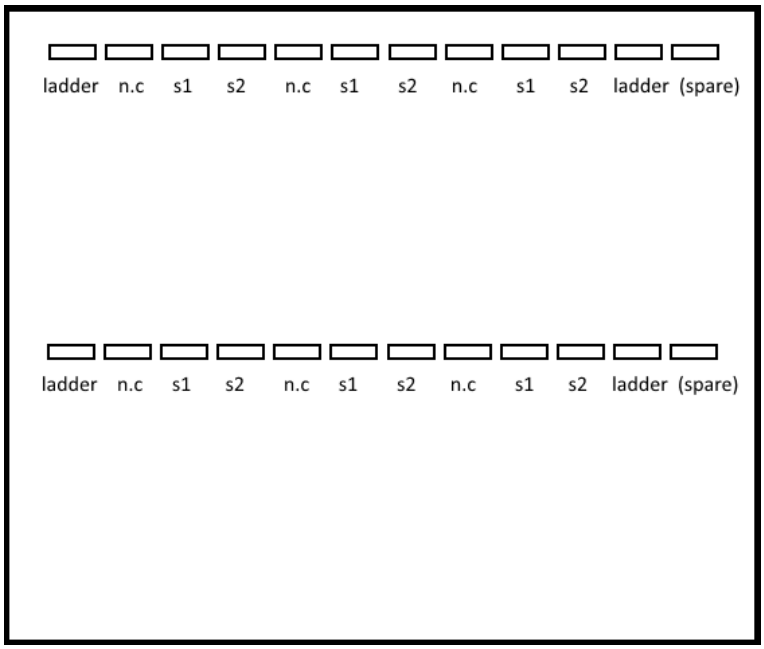
As it is inaccurate to pipette less than 1µl, make up a working stock that contains 4x (3 samples, plus extra for pipette loss) the volume of each component needed (but do NOT add DNA from the samples to this working stock!).

2. Place your samples in the PCR machine. We will need to process the samples **for the entire class at the same time** using the PCR program "Spider CO1. The program is given below and requires around 120mins to complete. The practical will resume once this has completed.

a. Heated lid to 103°C
b. 94°C for 180seconds
c. 40 cycles of:
    15seconds @94°C (denaturation)
    30seconds @50°C (annealing)
    40seconds @72°C (elongation)
d. 300seconds @72°C
e. Store at 12°C (manual removal)

3. Once you have recovered your samples from the PCR machine, you must now run some of each sample along an agarose gel to quantify the size of the fragments of DNA that you extracted.

Use 2µl Orange G dye and 5µl DNA PCR product in each well. In the wells marked “ladder”, add 2.5µl of the blue 6x dye and GeneRuler 100bp mixture. Arrange samples in the gels as follows:



Key: n.c = negative control s1 = species 1 s2 = species 2

When your gel has been filled, run the group gel (requires 15-20mins). After the gel has run, use the UV visualiser to determine whether you have extracted DNA of the appropriate length (~650bp). **Save the photograph of the gel under UV for your records.**

4. If the samples have yielded usable DNA, you can then prepare cleaned up COI DNA from the PCR product (If your sample did not produce workable DNA, then you can continue this stage using one of the "class samples" which was prepared as a backup and has viable DNA).

It is not enough, however, to simply extract some DNA of the right length – you need to make sure you have **enough** DNA to produce workable samples for sequencing. To do this you need cleaner COI samples. ‘Exo-star 1 step’ is one of many compounds that are used to remove excess primers and unincorporated dNTPS.

For each sample you will need to prepare (in a fresh labelled 0.2ml Eppendorf):

2µl Exo-star 1-step
10µl of your PCR sample

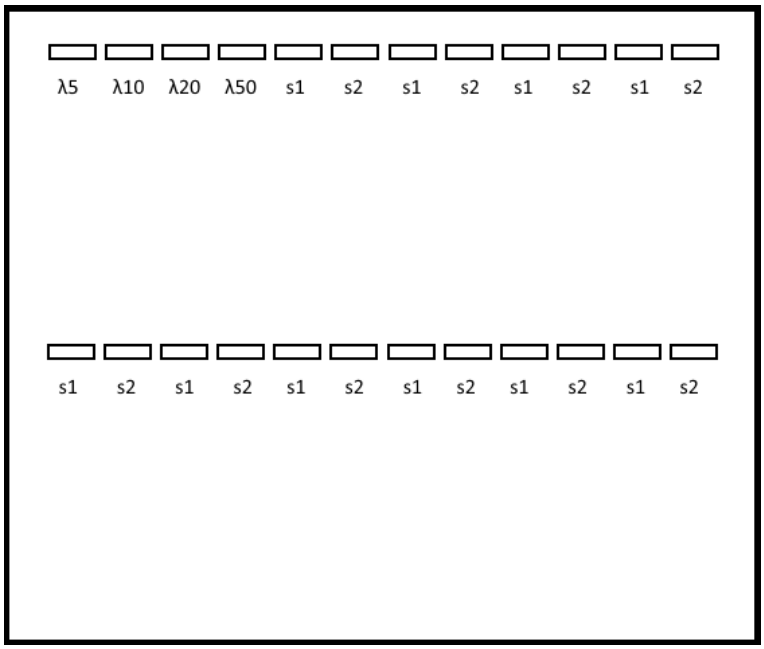
5. Run these samples in the thermocycler using the setting "37&80" with “heated lid = on”. This will take approximately 30minutes. **We will try to coordinate this step so that multiple samples can be run at the same time.**

6. After retrieving your sample from the thermocycler, run your digest DNA sample across another gel against 4 reference DNA concentrations (Lambda DNA; see below). For sequencing you need the DNA sample tubes to contain between 5-20ng of DNA.

Each DNA sample (from your own spider DNA extractions) should be prepared in a new 0.2ml Eppendorf with the following mix:

5µl Exo-star 1 step DNA sample
2µl Orange G dye

The lambda DNA is provided in four concentrations - 5, 10, 20, and 50ng/µl, and is provided pre-mixed with Orange G dye. Fill the gels (7µl) as follows: note that each gel can take 10 student's samples.



Key: λ = lambda DNA standard s1 = species 1 s2 = species 2

Examine the gel using the UV visualiser as before - the intensity of DNA fluorescence depends on the concentration (ng/µl) – higher concentrations of DNA glow brighter. **Determine the approximate amount of DNA in your extractions, compared with the standards, and save an annotated photograph of your gel under UV for your notes**.

The DNA in your sample will probably need to be diluted for Sanger sequencing (based on the results above). **Every sample sent for sequencing must have between 5-20ng of DNA and contain 6µl in total**. **There will always need to be 1µl of only a single primer in each sample (so you will end up with 4 sample tubes in total - each species has 1 tube with only the forward primer and 1 tube with only the reverse primer).** Note that concentration of the primers for this step (6.4µm/µl) is lower than for our initial extractions.

The dilution depends on the amount of DNA extracted. **Prepare each sample using the 0.2ml Eppendorf strips** (not loose tubes). The demonstrators will assign you a set of tubes which have been pre-numbered.

Construct a table similar to that below for your notes (given values are examples – you should calculate the volumes required from your own DNA samples observed on the gel).

Sample code	DNA concentration (estimated from gel)	Volume of H_2O required	Volume DNA required	Primer (conc.= 6.4µM/µl)	Final DNA amount for sequencing
1	~50ng/µl	4.5µl	0.5µl	1µl Forward (LCO1490)	~25ng
2	~20ng/µl	4µl	1µl	1µl Reverse (CHR-2)	~20ng
3	~10ng/µl	3µl	2µl	1µl Forward (LCO1490)	~20ng
4	~5ng/µl	1µl	4µl	1µl Reverse (CHR-2)	~20ng

7. Once your samples are labelled and packaged, the technicians will arrange for postage to the facility that carries out the Sanger sequencing. Turnaround is usually less than one week.

Appendix i. Anatomy of a typical spider

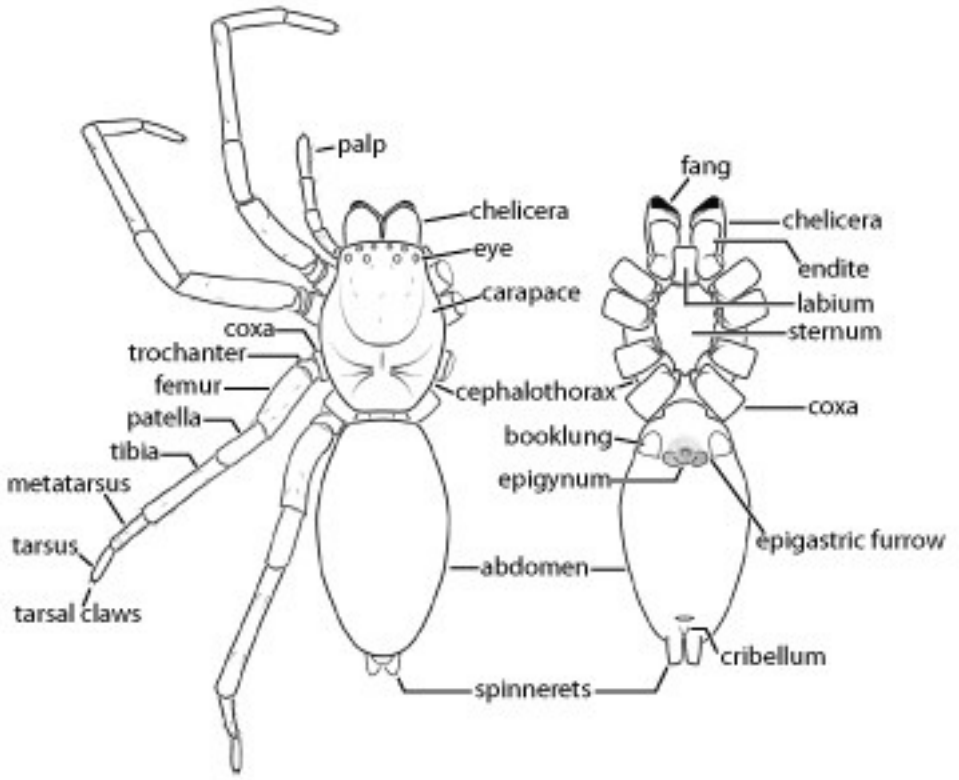


Figure 1. The typical spider body plan, with major structures labelled (Emerton 1902, *The common spiders of the United States*).

The palps are located near the chelicerae (mouthparts) – in adult males these are expanded, sclerotised and differentiated into often quite complex structures. Females will have simple palps that are not greatly expanded with a uniform surface.

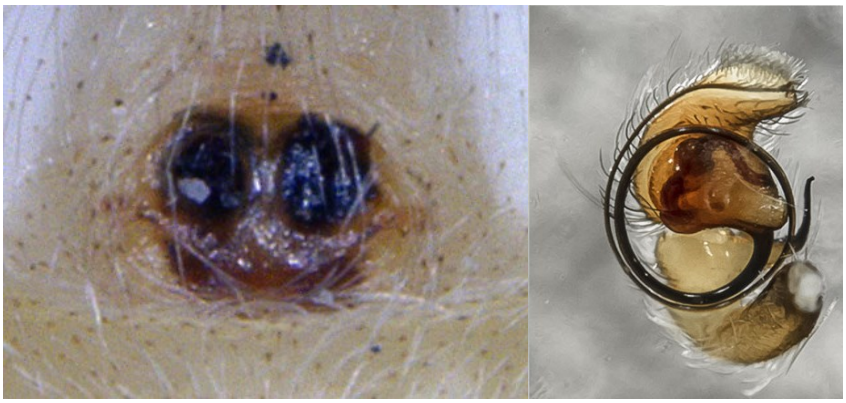


Figure 2. A female epigynum (left) and male palp (right; ectal view). Image of palp (cropped) copyright Robert Whyte (2014; https://commons.wikimedia.org/wiki/File:Afraflacilla-palps-whyte-A-Field-Guide-to-Spiders-of-Australia.jpg), shared under CC BY-SA 4.0.

Appendix ii: Assessment

The assessment for this practical has 2 parts:

1. Assemble your sequences in FASTA format from the sequence chromatograms **(80%).**
2. Search for your sequences using BLAST and interpret how successful your extractions were **(20%).**

Your final file upload to MMS should have the following format:

1. A page with the specimen names, collection localities and sequence data for both your specimens. E.g.:

Species name: *MySpecies one*
Collection label details: Example location, St Andrews. *Etc.*
Sample ID: *my_sample_number*

FASTA sequence:

```
>MySpecies cytochrome oxidase subunit 1 (COI) gene, mitochondrial <length
in bps>
aaGTTTATATTTTATTTTTGGAGCATGGGCTGCTATAGTAGGGACAGCAATAAGAGTATTAATTCGAATT
GAGTTAGGGCAAGTTGGAAGTTTGTTGGGGGATGATCAATTATATAATGTTATTGTTACTGCTCATGCTT
TTGTAATAATTTTTTTCATAGTTATACCTATTTTAATTGGAGGATTTGGTAATTGGTTAGTTCCTTTGAT
GTTAGGAGCCCCTGATATAGCTTTTCCTCGTATaAATAAtTTAAGATTTTGGTTA etc.
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2. A short comment (less than one side of A4) synthesising the morphological and sequence data you obtained for your two species.

Appendix iii: Interpreting a Sanger sequence chromatogram

The sequence data will be returned from the Sanger Sequencing centre as an .AB file (**there will be a separate one for both your forward and reverse primers, for each species**). The file name will be the code you were given for your samples in the practical (e.g. "**137**").

You can open these files with a free program called "FinchTV" (http://www.geospiza.com/ftvdlinfo.html) which is available via AppsAnywhere on all university computers.

When you open a sequence chromatogram you will see the a printout of base identities (A,T,G,C) along the top, with the read frequency (histogram) below (figure 1) – you will need to have a look through the sequence to make sure that the highest peaks match the allocated base. There is a good overview of interpretation of sequence chromatograms here: https://seqcore.brcf.med.umich.edu/sites/default/files/html/interpret.html

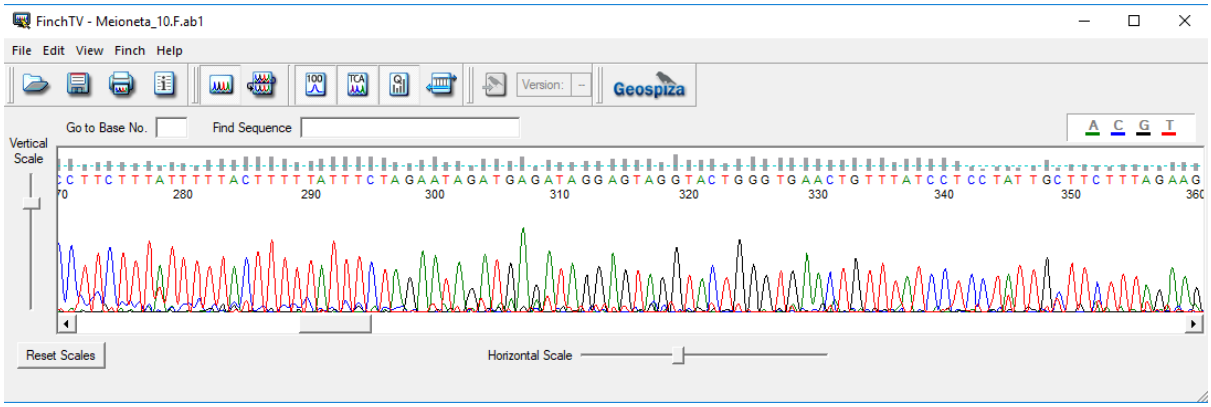

Figure 1: The GUI of FinchTV with a sequence chromatogram loaded. Different colours correspond to the frequencies of different bases (A, G, C, T) at each position in the chromatogram. The grey blocks at the top of the chromatogram indicate the quality of the read at each position.

How to use FinchTV to read your sequence file (windows PC):

1. **Open two copies of FinchTV.** You need two windows open so you can display both your forward and reverse primer read at the same time.
2. **On the reverse primer read, go to *View>reverse compliment*.** This flips your primer read about the horizontal axis so you can compare it with the forward read.
3. **Find your start primer region on the forward read.** You can do this by pasting the reverse compliment of the reverse primer sequence into the *find sequence* box and pressing enter.
4. **Find your end primer region on the forward read.** You can do this by pasting the forward primer into the *find sequence* box and pressing enter.
5. **Repeat this process on the reverse read to find your start and end points.**

The forward compliment of the forward primer should be found at the beginning of the reverse read. The reverse compliment of the reverse primer will be found at the end of the forward read. If you cannot find the whole primer (perhaps because your sequence is not high quality), try searching for a smaller segment of the primer. Sometimes you may need to search by eye to find distinct segments that belong to your primer.

LCO1490 (forward primer): GGTCAACAAATCATAAAGATATTGG
CHR-2 (reverse primer): GGATGGCCAAAAAATCAAAATAAATG

6. As you find your primer regions, go to *View>replace with X's* to mark the ends of your sequence read (we are not interested in reporting the primers with our sequence as we already know what these are; figure 2).

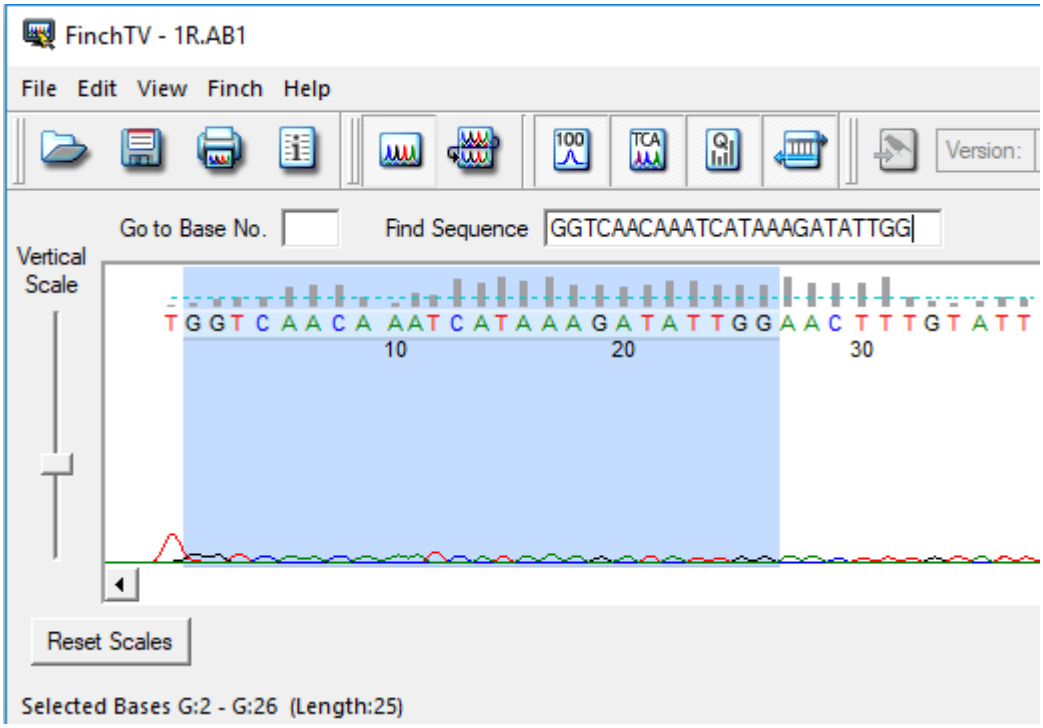

Figure 2: A reverse primer read, with the forward primer highlighted. This region can now be converted to X's to mark one end of the primer region of interest. The direction of the read is given in the file name – 1R (species 1, reverse primer read).

7. Working between the primer regions, you can generate your forward and reverse reads. You can copy and paste directly from the program into notepad to compare them, or compare visually using the two windows in finchTV. If you have a pair of good quality reads then the easiest way to check they match is to select all the bases between your primer ends on the forward read and paste this into the *find sequence* box on the reverse read. If you find the complete sequence, then you know both reads match for this region.

8. **FASTA sequences are written in capital letters when both your forward and reverse sequence are in agreement** – if you cannot read a base identity in both directions, then you should notate this using standard ambiguity codes (in red text for clarity) found here: http://www.dnabaser.com/articles/IUPAC%20ambiguity%20codes.html

For example:
WWGTTTATATTTTATTYNTGGAGCATGGG etc.

Remember, the COI region for barcoding purposes is 658bp in length, and chromatograms lose resolution the further along they go – so if you obtain a clean 658bp section of COI, there is no need to agonise over the identity of the bases >>700bp.

Technician's Notes

The practical runs over two sessions (9am - 1pm, 9am – 5pm). Students will work on their own samples, but will share equipment in pairs or groups of 4.

Day 1 – Spider identification and sample preparation equipment

Each group of 4 students should be provided with:

- 8 x Spider samples (stored @ -80°C)
- Identification keys (Roberts 1985; Roberts 1987)
- 4 x Glass beads in small beakers (10ml)
- 4 x Ink wells/small petri dishes
- 4 x Dissecting microscopes and lighting
- 4 x Fine size 4 forceps
- 4 x Storkbill forceps
- 8 x 1.5ml Eppendorf tubes - autoclaved
- Incubator @ 55°C
- 8 x Plastic pipettes
- 8 x Tungsten needles
- 8 x Glass slides to cut samples on
- 4 x 70% Ethanol in wash bottles
- 4 x 96% Ethanol in wash bottles for storing left over samples
- 4 x Protocol handouts
- White roll and waste bowl
- 4 x P200 Gilsons and pipette tips

In addition, the classroom should have the following equipment:

- Igloos – 1 per 8 students in class
- Fine marker pens
- Weigh balances

Chemicals

Each group of 4 students will require:

- 4 x 250µl Fly squishing buffer with Proteinase K (1:100 addition @ 20mg/ml)

We provided the FSB to students individually in 1.5ml Eppendorfs to minimise the risk of cross-contamination between samples.

Day 2 - Preparation of barcodes equipment

Each group of 4 students should be provided with:

- Heat block @ 95°C
- 4 x 25 sterile 0.2ml Eppendorfs in glass beakers
- 4 x P10/P20 & P100 Gilsons and tips
- 2 x Agarose gels (1.5% in TBE (0.75g in 50ml) plus Ethidium bromide)
- 2 x Gel rigs and power packs
- 4 x PCR strips (cut into strips of 4)
- 4 x PCR racks

In addition, the classroom should have the following equipment:

- Igloos – 1 per 8 students in class
- Tip disposal beakers
- PCR thermocycler
- UV Transilluminator

Chemicals

Each student will require:

- 2.5µl LCO1490 Forward primer @50µM
- 2 x 2.5µl LCO1490 Forward primer @ 6.4µM
- 2.5µl CHR-2 Reverse primer @ 50µM
- 2 x 2.5µl CHR-2 Reverse primer @ 6.4µM
- 200µl Clean H_2O
- 40µl Bioline myTAQ
- 50µl Orange G dye
- 10µl Illustra Exostar 1-step
    - The above 6 are to be aliquoted into 0.2ml eppendorfs and placed in PCR rack and kept on ice. 6.4µM primers to go out into igloos for second gel.

In addition, each gel station will require:

- 16µl Thermoscientific 100bp GeneRuler/6x dye mix
- Lambda DNA, 6 x 4 Standards (5, 10, 20 & 50µg/ml) 5 µl loaded plus 2µl orange G

All aliquots are to be kept on ice during the practical.

Formulations

Orange G Dye
10mM Tris HCl (pH to 7.6)
0.15% Orange G
60% Glycerol
60mM EDTA

Fly Squishing Buffer
1ml, 1M Tris HCl pH 8.2
0.5ml, 0.5M EDTA
0.5ml, 5M NaCl
Make to 100ml with clean H_2O
Autoclave
Add Proteinase K before aliquoting (1:100 @ 20mg/ml).

TBE 5X (2 Litres)
108g Tris HCL
55g Orthoboric acid
7.44g EDTA
Make up to 2 litres with de-ionised water and stir.
Dilute 1:10 to use (0.5X)

Purchasing

This list includes necessary purchases from external suppliers, and approximate costs for each are given (as of 2018).

Illustra exostar-1 step (pack size: 100 reactions)
Item code: 12124082
Fisher scientific UK limited
£67

Strips of 8 PCR tubes, with detached flat cap strips (pack size 250)
Item code: 732-1520
VWR INTERNATIONAL LTD
£189.12

0.2ml thin PCR tube flat cap
Item code: 11376044
FISHER SCIENTIFIC UK LIMITED
£23.32

- This design of PCR tube and flat cap is required for sequencing at Edinburgh; a pack of this size will last more than one run of the practical.

Mytaq mix, 200 x 50ul reactions (pack size 1)
Item code: BIO-25041

BIOLINE REAGENTS
£91.20

- Due to pipetting loss, in our experience each tube of mytaq will yield ca. 30 aliquots of 40µl.

Lambda DNA (pack size 250ug)
Item code: D1501
PROMEGA
£61.20

Oligo's X 4
CHR-2 and LCO1490 sequences
INTEGRATED DNA TECHNOLOGIES INC
£11.02

Proteinase k (pack size 100mg)
Item code: BIO-37037
BIOLINE REAGENTS
£92.40

Gene ruler 100bp plus DNA ladder (pack size 5 x 50ug)
Item code: SM0322
FISHER SCIENTIFIC UK LIMITED
£186.84

- A pack of this size will last more than one run of the practical.

List of DNA barcodes generated during the session (2016)

Key: Uppercase = sequences read using both forward and reverse primers and in agreement between both reads. Lowercase = sequences read in only a single direction. N = bases which could not be resolved from either chromatogram read.

>Bathyphantes gracilis cytochrome oxidase subunit I gene, partial cds; mitochondrial 611bp forward read only
aactttatatttaatttttggtgcttgagcagctatagttgggactgctataagagttttaattcgaattgaattggggcaaactggaagaatatta
ggagatgaccatttatataatgtcattgttactgcacatgcttttgttataatttttttttatagtgatacctattttgattgggggtttggtaattggtt
ggttcctttaatattaggggctccagatatagcttttcctcgaatgaataatttaagattttggttactccctccttcattattaatattatttatttctt
caatagtagaaataggagttggagcaggttgaactgtttatcctcctttagcttctttagaagggcattcagggagatctgtggattttgctatttttt
tctttacatttagctggggcttcgtcaattataggggctattaattttatttcgacaattataaatatacgagcttatgatatcagaatagaaaaggt
ttctttatttgtttgatcagtattgattactgcagttttattattattatctttacctgttttagcaggagctattactatattattaactgatcgaaattt
taatacttcttttt

>Cryphoeca silvicola cytochrome oxidase subunit I gene, partial cds; mitochondrial 648bp
gactttgtatttggtttttgggtCTTGATCTGCTATAGTAGGAACTGCTATAAGAGTTTTAATTCGAATTGAATTAGGTCA
GCCTGGGAGATTTTTAGGGGATGATCATTTATATAATGTAATTGTAACTGCACATGCtTTTATTaGATTTTTTTta
tagtaaTACCTATTTTGATTGGGGGTTTTGGAAATTGATTAATTCCTTTAATATTAGGTGCTCCTGACATAGCTTT
TCCTCGTATAAATAATTTAAGATTTTGACTTTTACCTCCTTCTTTAATTTTATTATTTATTTCTTCAATAGTAGAGA
TGGGAGTAGGTGCTGGGTGAACTATTTATCCACCATTAGCTTCTATAGTAGGACATAATGGGAGATCTGTGGA
TTTTGCTATTTTTTCTTTACATTTAGCTGGGGCTTCTTCAATTATAGGGGCGATTAATTTTATTTCTACTATTTTAA
ATATACGATATTATGGAATAAgaatagagaagattcctttatttgtttgatcagttttaattacagcggtattattacttttatctttacctg
ttttagcaggtgctattacaatattgttaactgatcggaattttaatacttctttttttgacccctctgggggtggggatcctattttatttcaa

>Diplocephalus latifrons cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtctatattttatttttggtgcatgggctgctatagtagggacagcaaTAAGAGTTTTAATTCGAATTGAGTTAGGTCAAACTG
GTAGATTGTTAGGTGATGATCAGTTATATAATGTTATTGTAACTGCTCATGCATTTGTTATAATTTTTTTTATAG
TGATACCTATTTTAATTGGGGGATTTGGAAATTGGTTAGTTCCTTTAATGTTAGGGGCTCCTGATATAGCTTTTC
CTCGAATAAATAATTTAAGTTTTTGGTTATTACCCCCCTCTTTGTTTTTATTGTTTATTTCTAGAATAGATGAAAT
AGGAGTTGGGGCGGGATGAACAGTGTATCCTCCTCTTGCCTCTTTAGAAGGGCATCCTGGAAGATCGGTAGA
TTTTGCTATTTTTTCTCTACACTTAGCCGGGGCATCGTCAATTATGGGGGCTATTAACTTTATCTCTACTATTTTA
AACATACGTGGGTATGGAATAACTATAGATAAGGTCCCTTTATTTGTTTGATCGGTTTTGATTACTGCTGTCTT
ATTACTTTTATCCTTACCAGTTTTAGCAGGAGCTATTACTatgcttttgacagatcgtaattttaatacttctttctttgacccttct
ggcgggggagatcctgttttatttcaa

>Diplocephalus latifrons cytochrome oxidase subunit I gene, partial cds; mitochondrial 566bp
aagtctatattttatttttggtgcatgggctgctatagtagggacagcaataagagttttaattcgaattgagttaggtcaaactggtagattgtta
ggtgatgatcagttatataatgttattgtaactgctcatgcatttgttataatttttttttatagtgatacctattttaattgggggtttggaaattggt
tagttcctttaatgttaggggctcctgatatagcttttcctcgaataaataatttaagtttttggttattacctccctctttgtttttattgtttatttctag
aatagatgaaataggagttggggcgggatgaacagtgtatcctcctcttgcctctttagaagggcatcctggaagatcagtagattttgctatttttt
ctctacacttagccggggcatcgtcaattatgggggctattaactttatctctactattttaaacatacgtgggtatggaataactatagataagg
tccctttatttgtttgatcggttttgattactgctgtcttattacttttatccttaccagttttagcaggag

>Erigona atra cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtttgtattttgtttttggggcttgggctgctATAGTGGGAACAGCAATAAGAGTATTAATTCGTATTGAGTTAGGACAA
ACTGGAAGATTGTTAGGGGATGATCAATTATATAATGTTATCGTTACGGCGCATGCTTTTATTATAATTTTTTTT
ATAGTTATACCTATTTTAATTGGGGGATTTGGCAACTGATTAGTTCCTTTAATATTAGGGGCTCCTGATATAGC
TTTTCCTCGTATAAATAATTTAAGATTTTGATTATTACCTCCTTCTTTATTATTATTATTTATCTCTAGTATAGATG
AGATAGGTGTGGGGGCGGGATGAACAATTTATCCTCCTCTAGCTTCTTTAGAGGGTCATTCTGGTAGTTCTGT
TGATTTTGCAATTTTTTCTTTACACTTAGCTGGTGCTTCTTCTATTATAGGGGCTATTAATTTTATTTCTACAATTT
TAAATATGCGTGGGTATGGAATAACTATAGAAAAAGTTCCTTTATTTGTATGGTCTGTTTTAATTACAGCTGTA
CTGTTATTATTATCTTTACCTGTGCTTGCAGGAgctatcactatgcttttaactgatcgaaattttaatacttctttttttgacccttctg
ggggggggtgatcctgtgttatttcaa

>Erigone arctica cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtttgtatttttgtttttggggcttgggctgctatagtgggaacagcaataagagtattaattcgtATTGAGTTAGGACAAACTGGAA
GATTGTTAGGGGACGATCAATTATATAATGTTATTGTTACGGCGCATGCTTTTATTATAATTTTTTTTATAGTTA
TACCTATTTTAATTGGGGGATTTGGTAATTGATTAGTTCCTTTAATATTAGGGGCTCCTGATATAGCTTTTCCTC
GTATAAATAATTTAAGATTTTGATTATTACCCCCTTCTTTATTATTATTATTTATCTCTAGAATAGATGAAATAGG
TGTGGGGGCGGGATGAACAATTTATCCTCCTCTAGCTTCTTTAGAGGGTCATTCTGGTAGTTCTGTTGATTTTG
CAATTTTCTCTTTACACTTAGCTGGTGCTTCTTCTATTATAGGGGCTATTAATTTTATTTCTACAATTTTAAATAT
GCGTGGGTATGGAATAACTATAGAAAAAGTTCCTTTATTTGTATGGTCTGTTTTAATTACAGCCGTATTGTTAT
TATTATCTTTACCTGTGCTTGCAGGAGTATTactatgcttttaactgatcgaaattttaatacttctttttttgatccttctggaggggggt
gatcctgtgttatttcaa

>Helophora insignis cytochrome oxidase subunit I gene, partial cds; mitochondrial 655bp
gagtttatattttgtatttggagcttgggctGCTATAGTTGGAACAGCAATAAGTGTATTAATTCGGATTGAGTTAGGTCAA
ACTGGAAGATTATTATTATTAGGAGATGATCAATTGTATAATGTAATTGTTACGGCTCATGCTTTTGTTATAATT
TTTTTTATAGTTATACCTATTTTGATTGGAGGATTTGGAAATTGGTTAGTTCCTTTAATATTAGGGGCTCCGGAT
ATGGCTTTCCCACGTATGAATAATTTAAGATTTTGACTTTTGCCACCTTCTTTATTTTTGTTATTTATTTCTAGAAT
AGATGAAATAGGTGTTGGGGCTGGGTGGACAGTTTATCCTCCACTTTCTTCTTTAGAGGGACATTCTGGAAGA
TCAGTGGATTTTGCTATTTTTTCTTTACATTTAGCTGGTGCTTCGTCTATTATAGGGGCCATTAATTTTATTTCTA
CTATTTTAAATATGCGAGGATATGGAATAACTATAGAAAAAGTTCCTTTATTTGTGTGATCGGTGTTAATTACG
GCTGTTTTATTACTTTTGTCATTACCGGTTTTAGCAGGTGCTATTACTATATTATTAACAGATCGTAATTTTAATA
CTTCATTTTTtgatccttcagggggaggtgatccaattttattccaa

>Hypomma c.f bituberculatum immature cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagcttatattttgtttttggtgcttgagctgctatGGTGGGAACAGGGATAAGAGTTTTAATTCGAGTTGAGTTGGGTCAG
GTTGGCAGTTTATTAGGCGATGATCAATTGTATAATGTTATTGTTACGGCTCATGCTTTTGTTATAATTTTTTTT
ATAGTTATACCTATTTTAATTGGAGGGTTTGGCAATTGGCTTGTTCCTCTAATGTTAGGTGCTCCGGATATAGC
TTTTCCTCGTATAAATAATTTGAgATTTTGATTGTTGCCTCCATCTTTATTATTATTATTTATTTCTAGAATGGATG
AGATAGGAGTAGGAGCTGGATGAACGGTATACCCTCCTCTTGCTTCTTTGGAGGGTCATTCTGGTAGATCAgT
ANATTTTGCTATTTTTTCTTTACATTTAgCGGGAGCGTCATCTATTATGGGAGCTATTAATTTTATTTCTACTATT
TTTAATATGCGGGGATATGGAATGACTTTAGAAAAAATTCCTTTATTTGTTTGATCTGTATTAATTACAGCTGTT
TTGTTGTTGTTATCTTTGCCTGTTCTAgCAGGGGCTATTACTATACTTCTTActgatcgaaattttaatacttctttttttgatc
cttctgggggggggagatcctgttttatttcaa

>Hypomma c.f bituberculatum immature cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagcttatattttgtttttggtgcttgagctgcTATGGTGGGAACAGGGATAAGAGTTTTAATTCGAGTTGAGTTGGGTCAG
GTTGGCAGTTTATTAGGCGATGATCAATTGTATAATGTTATTGTTACGGCTCATGCTTTTGTTATAATTTTTTTT
ATAGTTATACCTATTTTAATTGGAGGGTTTGGCAATTGGCTTGTTCCTCTAATGTTAGGTGCTCCGGATATAGC
TTTTCCTCGTATAAATAATTTGAGATTTTGATTGTTGCCTCCATCTTTATTATTATTATTTATTTCTAGAATGGAT

GAGATAGGAGTAGGAGCTGGATGAACGGTATACCCTCCTCTTGCTTCTTTGGAGGGTCATTCTGGTAGATCAG
TAGATTTTGCTATTTTTTCTTTACATTTAGCGGGAGCGTCATCTATTATGGGAGCTATTAATTTTATTTCTACTAT
TTTTAATATGCGGGGATATGGAATGACTTTAGAAAAAATTCCTTTATTTGTTTGATCTGTATTAATTACAGCTGT
TTTGTTGTTGTTATCTTTGCCTGTTCTAGCAGGGGCTATTACTATACTTCTTACTGATCGAAATTTTAATACTTCT
TTTTttgatccttctgggggggagatcctgttttatttcaa

>Linyphia c.f hortensis immature cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aactttatattttatttttggggcgtgagctgcaatagtaggaacagctataagagttttaattcgaattgagttaggaCAGACAGGGAGTA
TATTAGGAGATGACCAGTTATATAATGTAATTGTCACTGCTCATGCTTTTGTAATAATTTTTTTTATAGTGATAC
CTATTTTAATTGGGGGGTTTGGAAATTGGTTAGTTCCTTTAATATTAGGGGCTCCCGACATAGCTTTTCCTCGT
ATAAATAATTTAAGTTTTTGGTTATTACCTCCTTCTTTGTTATTATTATTTATTTCATCAATGGCTGAAATGGGGG
TAGGGGCAGGTTGAACTGTTTACCCTCCTCTTTCTTCTTTAGAAGGGCATTCAGGAAGATCTGTTGATTTTGCT
ATTTTTTCTCTGCATTTAGCTGGGGCTTCTTCAATTATAGGTGCTATTAATTTTATTTCTACTATTTTAAATATAC
GAGCTTTTGGTGTTAGTATAGAAAAGGTTTCTTTGTTTGTATGATCAGTTTTAATTACTGCGGTTTTATTATTAT
TATCTTTACCTGTTTTAGCAGGGGCTATTACTATATTGTTGACTGATCGAAATTTTAATACATCTTTTTTTGAccct
gcaggggtggggatccaattttatttcaa

>Linyphia c.f hortensis immature cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aactttatattttatttttggggcgtgagctgcaatagtaggaacagctataagagttttaattcgaatTGAGTTAGGACAGACAGGGA
GTATATTAGGAGATGACCAGTTATATAATGTAATTGTCACTGCTCATGCTTTTGTAATAATTTTTTTTATAGTGA
TACCTATTTTAATTGGGGGGTTTGGAAATTGGTTAGTTCCTTTAATATTAGGGGCTCCCGACATAGCTTTTCCTC
GTATAAATAATTTAAGTTTTTGGTTATTACCTCCTTCTTTGTTATTATTATTTATTTCATCAATGGCTGAAATGGG
GGTAGGGGCAGGTTGAACTGTTTACCCTCCTCTTTCTTCTTTAGAAGGGCATTCAGGAAGATCTGTTGATTTTG
CTATTTTTTCTCTGCATTTAGCTGGGGCTTCTTCAATTATAGGTGCTATTAATTTTATTTCTACTATTTTAAATATA
CGAGCTTTTGGTGTTAGTATAGAAAAGGTTTCTTTGTTTGTATGATCAGTTTTAATTACTGCGGTTTTATTATTA
TTATCTTTACCTGTTTTAGCAGGGGCTATTACTATATtgttgactgatcgaaattttaatacatctttttttgaccctgcaggggtg
gggatccaattttatttcaa

>Microneta viaria cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtttatattttgtatttggggcttggtctGCGATAGTTGGAACGGCAATAAGAGTATTAATTCGAGTTGAGTTAGGTCA
AGTTGGTAGTTTGTTAGGAGATGACCAATTATATAATGTAATTGTTACGGCTCACGCTTTTGTTATAATTTTTTT
TATAGTGATACCAATTTTAATTGGGGGATTTGGTAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATATAG
CTTTCCCTCGGATAAATAATTTGAGATTTTGACTTTTACCCCCCTCTTTATTTTTATTGTTTATTTCAAGTATAGAT
GAGATAGGGGTAGGGGCAGGTTGGACAGTTTATCCTCCTCTTTCTTCATTAGATGGGCATTCGGGGAGTTCAG
TGGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCATCTTCTATTATGGGGGCTATTAATTTTATTTCTACTAT
TTTAAATATACGAGGGTATGGGATAAGCATAgATAGGGTTCCTTTATTTGTTTGGTCTGTATTAATTACGGCTG
TTCTTTTATTGTTATCTTTGCCAGTGTTAGCAGGGGCGATTACnATGTTGTTAACAGATCGAAATTTCAATACAT
CATtttttgatcctgctggaggggggatcctgttttgtttcaa

>Microneta viaria cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtttatattttgtatttggggcttggtctgcgataGTTGGAACGGCAATAAGAGTATTAATTCGAGTTGAGTtAGGTCAAG
TTGGTAGTTTGTTAGGAGATGATCAATTATATAATGTAATTGTTACGGCTCACGCTTTTGTTATAATTTTTTTTA
TAGTGATACCAATTTTAATTGGGGGATTTGGTAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATATAGCT
TTCCCTCGGATAAATAATTTGAGATTTTGACTTTTACCCCCCTCTTTATTTTTATTGTTTATTTCAAGTATAGATG
AGATAGGGGTAGGGGCAGGTTGGACAGTTTATCCTCCTCTTTCTTCATTAGATGGGCATTCGGGGAGTTCAGT
GGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCATCTTCTATTATGGGGGCTATTAATTTTATTTCTACTATT
TTAAATATACGAGGGTATGGGATAAGCATAGATAGGGTTCCTTTATTTGTTTGGTCTGTATTAATTACGGCTGT
TCTTTTATTGTTATCTTTGCCAGTGTTAGcaggggcgattacgatgttgttaacagatcgaaatttcaatacatcatttttgatcctgc
tggaggggggatcctgttttgtttcaa

>Microneta viaria cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aagtttatattttgtatttggggcttggtctgcgataGTTGGAACGGCAATAAGAGTATTAATTCGAGTTGAGTTAGGTCAAG
TTGGTAGTTTGTTAGGAGATGATCAATTATATAATGTAATTGTTACGGCTCACGCTTTTGTTATAATTTTTTTTA
TAGTGATACCAATTTTAATTGGGGGATTTGGTAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATATAGCT
TTCCCTCGGATAAATAATTTGAGATTTTGACTTTTACCCCCCTCTTTATTTTTATTGTTTATTTCAAGTATAGATG
AGATAGGGGTAGGGGCAGGTTGGACAGTTTATCCTCCTCTTTCTTCATTAGATGGGCATTCGGGGAGTTCAGT
GGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCATCTTCTATTATGGGGGCTATTAATTTTATTTCTACTATT
TTAAATATACGAGGGTATGGGATAAGCATAGATAGGGTTCCTTTATTTGTTTGGTCTGTATTAATTACGGCTGT
TCTTTTATTGTTATCTTTGCCAGTGTTAGCAGGGGCGATTACGATGTTGTTAACAGATCGAAATTTCAATACATC
atttttgatcctgctggaggggggatcctgttttgtttcaa

> Microneta viaria cytochrome oxidase subunit I gene, partial cds; mitochondrial 650bp
aagtttatattttgtatttggggcttggtctgcgatagttggaacggcaaTAAGAGTATTAATTCGAGTTGAGTTAGGTCAAGTTG
GTAGTTTGTTAGGAGATGATCAATTATATAATGTAATTGTTACGGCTCACGCTTTTGTTATAATTTTTTTTATAG
TGATACCAATTTTAATTGGGGGATTTGGTAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATATAGCTTTC
CCTCGGATAAATAATTTGAGATTTTGACTTTTACCCCCCTCTTTATTTTTATTGTTTATTTCAAGTATAGATGAGA
TAGGGGTAGGGGCAGGTTGGACAGTTTATCCTCCTCTTTCTTCATTAGATGGGCATTCGGGGAGTTCAGTGGA
TTTTGCTATTTTTTCTTTACATTTAGCTGGGGCATCTTCTATTATGGGGGCTATTAATTTTATTTCTACTATTTTAA
ATATACGAGGGTATGGGATAAGCATAGATAGGGTTCCTTTATTTGTTTGGTCTGTATTAATTACGGCTGTTCTT
TTATTGTTATCTTTGCCAGTGTTAGCAGGGGCGATTACGATGTTGTTAACCagatcgaaatttcaatacatcatttttgat
cctgctggaggggggatcctgttttgtttcaa

> Microneta viaria cytochrome oxidase subunit I gene, partial cds; mitochondrial 654bp
aagtttatattttgtatttggggcttggtctgCGATAGTTGGAACGGCAATAAGAGTATTAATTCGAGTTXAGTTAGGTCAA
GTTGGTAGTTTGTTAGGAGATGATCAATTATATAATGTAATTGTTACGGCTCACGCTTTTGTTATAATTTTTTTT
ATAGTGATACCAATTTTAATTGGGGGATTTGGTAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATATAGC
TTTCCCTCGGATAAATAATTTGAGATTTTGACTTTTACCCCCCTCTTTATTTTTATTGTTTATTTCAAGTATAGAT
GAGATAGGGGTAGGGGCAGGTTGGACAGTTTATCCTCCTCTTTCTTCATTAGATGGGCATTCGGGGAGTTCAG
TGGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCATCTTCTATTATGGGGGCTATTAATTTTATTTCTACTAT
TTTAAATATACGAGGGTATGGGATAAGCATAGATAGGGTTCCTTTATTTGTTTGGTCTGTATTAATTACGGCTG
TTCTTTTATTGTTATCTTTGCCAGTGTTAGCAGGGGCGATTAcGATGAcGATGtTGTTAACAGATCGAAATTTCA
ATacatcatttttgatcctgctggaggggggatcctgttttgtttcaa

>Monocephalus fuscipes cytochrome oxidase subunit I gene, partial cds; mitochondrial 608bp
aacattatattttattttgctttatgggcaggaactttaggagcttcaataagaataattattcgtttagaattaagttctcctggggctttaattaa
taatgatcaaatttataatacaattattacagcccatgcctttattataatttttttatagttataccttcctagttggaggatttggaaattgatta
atcccattaatattaggagtgcctgatatagcttttcctcgaataaataatataagattttgattactccctccttctttatttttattaattttaagaa
attttattggaacgggtgtaggaaccggatgaactttatatcctccttttatcatctattgttggacatgattcaccttctgtagatttaggaatttttc
tatccatattgctggaatttcctcaattataggatcaattaattttattgttactattttaaatatacacacaaaaactcattnnnntaattttcttcc
tttattcacatgatcaattttaattacagcaattcttcttctgttatcattaccagttcttgcaggagcaattactatacttcttacagatcgaaatctt
aatacatctt

>Monocephalus fuscipes cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
tagtttatactttatttttggggcttgggctgcaATAGTAGGAACAGCAATAAGAGTATTAATTCGAGTTGAGTTAGGGCAA
ACTGGTAGCTTGTTGGGGGATgATCAGTTATATAATGTTATTGTTACTGCACATGCCTTTGTGATAATTTTTTTT
ATAGTTATGCCTATTTTAATTGGGGGGTTTGGAAATTGATTAGTACCATTAATATTAGGGGCTCCTGATATAGC
CTTTCCTCGTATAAATAATTTGAGATTTTGGCTATTACCCCCTTCTTTGTTATTATTATTTATTTCAAGTATAGAT
GAAATAGGGGTCGGAGCAGGTTGAACTGTGTATCCTCCTCTTGCTTCTTTAGAAGGACATTCTGGTAGCTCAG
TAGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCTTCATCCATTATAGGGGCTATTAATTTTATTTCTACTAT
TTTTAATATACGAGGGTATGGTATGACTATAGAGAAGGTTCCATTATTTGTGTGGTCTGTTTTAATTACAGCTG

TTTTGTTACTTTTGTCTTTACCTGTGTTAGCGGGGGCTATTACAATGCTTTTAACTGATCGtAATTTTAATACATC
ATTTTTtgatccgtctgggggaggggacccagtgttatttcaa

>Oedothorax retusus cytochrome oxidase subunit I gene, partial cds; mitochondrial 623bp
gatctactatggtgggtacagctatgagagttttgattcgggttgagttaggtcaaactggtagtttattgggggatgaccagatgtataatgttat
tgttacttctcatgcttttgttatgatttttttttatagtgatacctattttaattgggggggtttggtaattggttagtgcctttaatgttaggtgctcctga
tatagcttttcctcgaataaataatttaagattttggttgttaccaccttctttgtttttattattcatttctagaatagatgagataggtgtgggtgcc
gggtggactatttatcctcctcttgcttctttagagggacattctggtagttcagtagattttgctatttttttctttacatttagctggggcttcatctat
tataggagctattaattttatttctactattttaaatatgcgggggtacgggataactatagaaaaagttcctttatttgtctggtcagtgttaattac
ggctgttttgttgttgttgtctttacctgtgttagcaggagctattactatactttaactgatcgtaattttaatacttctttttttgacccttctgggggg
gggggatcctgttttgtttcan

>Porrhomma pygmaeum cytochrome oxidase subunit I gene, partial cds; mitochondrial 611bp
ttggaacggctataagagtattaattcgagttgagttaggacagacagggagaatattaggagatgatcatttatataatgttattgtaactgctc
atgcatttgttataatttttttttatggttatacctattttaattgggggatttgggaattgattagtacctttaatgttaggggctcctgatatagcattt
ccacgtataaataatttaagtttttgacttttacctccttcgttatgtatgctatttatatcttctatagtagaaataggtgttggagcaggatgaact
atttatcctccttttggcttctttagaagggcatgggggaagttctgtagattttgctatttttttctttacatttagcaggtgcttcttctattataggggc
aattaattttatttctacaattttaaatatacgagttaataatattagtatagaaagggtttctttgtttgtgtgatctgttttaattactgcagtgttat
tgcttttgtctttacctgttttagcaggggctattactatattattaactgatcgaaattttaatacttctttttttgatccagcaggggaggagatcc
tgttttatttcaa

>Robertus lividus cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
aactttatatttgatttttggggtgtgatcagctatagttggtacaGCTATGAGTGTCCTAATTCGGGTAGAATTAGGACAATCT
GGAAGATTGTTAGGGGATGATCAATTATATAATGTAATTGTAACTGCTCATGCTTTTGTAATAATTTTTTTTATA
GTAATACCTATTATAATTGGGGGGTTTGGAAATTGATTAGTTCCTTTAATGTTAGGGGCTCCAGATATAGCTTT
CCCTCGAATAAATAATTTAAGATTTTGATTATTACCTCCTTCTTTATTTCTATTATTTATTTCTTCTATAGTTGAAA
TAGGAGTAGGTGCTGGGTGAACAATTTATCCTCCTTTATCTTCTTTAGAGGGTCATTCAGGAAGATCAGTTGAT
TTTGCGATTTTTTCTCTTCATCTTGCAGGTGCTTCTTCTATTATAGGGGCTGTAAATTTTATTTCTACTATTTTAAA
TATACGTGTGTATGGAATATCTATAGAAAAAGTTACTTTATTTGTATGATCAGTTTTAATTACTGCTATTCTTTT
ATTATTATCGTTACCTGTTTTAGCAGGAGCTATTACTATGTTATTAACTGATCGAAATTTTAATACTTCTTTTTTT
gatccttctggaggaggagatccaattttatttcaa

>Robertus lividus cytochrome oxidase subunit I gene, partial cds; mitochondrial 626bp
gtgatcacactatagttggtacagctatgagtgtcctaattcgggtagaattaggacaatctggaagattgttaggggatgatcaattatataatg
taattgtaactgctcatgcttttgtaataatttttttttatagtaatacctattataattgggggggtttggaaattgattagttcctttaatgttaggggc
tccagatatagctttccctcgaataaataatttaagattttgattattacctccttctttatttctattatttatttcttctatagttgaaataggagtag
gtgctgggtgaacaatttatcctccttttatcttctttagagggtcattcaggaagatcagttgattttgcgatttttttctcttcatcttgcaggtgcttct
tctattataggggctgtaaattttatttctactattttaaatatacgtgtgtatggaatatctatagaaaaagttactttatttgtatgatcagttttaa
ttactgctattcttttattattatcgttacctgttttagcaggagctattactatgttattaactgatcgaaattttaatacttctttttttgatccttctgg
aggaggagatccaattttatttcaa

>Robertus lividus cytochrome oxidase subunit I gene, partial cds; mitochondrial 616bp
tatagttggtacagctatgagtgtcctaattcgggtagaattaggacaatctggaagattgttaggggatgatcaattatataatgtaattgtaact
gctcatgcttttgtaataatttttttttatagtaatacctattataattgggggggtttggaaattgattagttcctttaatgttaggggctccagatatag
ctttccctcgaataaataatttaagattttgattattacctccttctttatttctattatttatttcttctatagttgaaataggagtaggtgctgggtga
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cttttattattatcgttacctgttttagcaggagctattactatgttattaactgatcgaaattttaatacttctttttttgatccttctggaggaggaga
tccaattttatttcaa

>Tenuiphantes cristatus cytochrome oxidase subunit I gene, partial cds; mitochondrial 650bp

aactttatattttatttttggagcttgagccgctatagtgggaactGCAATAAGAGTATTAATTCGAATCcGAGTTAGGACAAAC
TGGAAGAATGTTGGGAGATGACCAATTATATAATGTAATTGTTACTGCTCATGCTTTTGTGATAATTTTTTTTAT
GGTTATACCTATTTTAATTGGAGGATTTGGAAATTGGTTAGTTCCTTTGATACTAGGGGCTCCTGATATAGCTT
TCCCACGAATAAATAATCTTAGATTTTGATTACTTCCTCCTTCTTTATTACTATTATTTATTTCAAGAATAGTTGA
GATAGGGGTTGGGGCAGGATGAACTGTTTATCCTCCTCTTGCTTCTTTAGAAGGACATGCCGGGAGTTCTGTA
GATTTTGCTATTTTTTCTCTTCACTTAGCTGGGGCATCTTCAATTATAGGAGCTATTAATTTTATCTCCACTATTG
TAAATATGCGAGGTTACGGAGTTTCAATAGAAAAGGTTCCTTTATTTGTGTGATCTGTTTTAATTACTGCTGTG
TTATTGTTGTTATCTCTTCCTGTATTAGCAGGAGCTATTACAATGTTATTAActgatcgaaattttaatacttctttttcgac
cctgcagggggagaggatcctgtattgtttcag

>Tenuiphantes cristatus cytochrome oxidase subunit I gene, partial cds; mitochondrial 648bp
aactttatattttatttttggagcttgagccgctatagtgggAACTGCAATAAGAGTATTAATTCGAATCGAGTTAGGACAAAC
TGGAAGAATGTTGGGAGATGACCAATTATATAATGTAATTGTTACTGCTCATGCTTTTGTGATAATTTTTTTTAT
GGTTATACCTATTTTAATTGGAGGATTTGGAAATTGGTTAGTTCCTTTGATACTAGGAGCTCCTGATATAGCTT
TTCCACGAATAAATAATCTTAGATTTTGATTACTTCCTCCTTCTTTATTACTATTATTTATTTCAAGAATAGTTGA
GATAGGGGTTGGGGCAGGATGAACTGTTTATCCTCCTCTTGCTTCTTTAGAAGGACATGCCGGGAGTTCTGTA
GATTTTGCTATTTTTTCTCTTCACTTAGCTGGGGCATCTTCAATTATAGGAGCTATTAATTTTATCTCCACTATTG
TAAATATGCGAGGTTACGGAGTTTCAATAGAAAAGGTTCCTTTATTTGTGTGATCTGTTTTAATTACTGCTGTG
TTATTGTTGTTATCTCTTCCTGTATTAGCAGGAGCTATTACAATGTTATTAACTGATGAAATTTTAATACTTCTTT
TTtcgatcctgcagggggaggggatcctgtattgtttcag

>Tenuiphantes flavipes cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
gactttatattttatttttggagcgtgagctGCTATAGTAGGAACTGCTATAAGAGTATTAATTCGGATTGAATTGGGGCA
AACTGGAAGAATACTGGGGGATGACCAGTTGTATAATGTTATTGTAACTGCACATGCTTTTGTAATAATTTTTT
TTATAGTTATACCTATTTTGATTGGAGGATTCGGTAATTGATTAGTTCCTTTAATACTAGGGGCTCCTGATATAG
CTTTTCCTCGTATAAATAATTTAAGATTTTGACTTTTACCCCCTTCTCTATTATTATTATTTATTTCAAGTATAGTT
GAGATGGGAGTTGGGGCAGGGTGGACAGTGTATCCGCCTCTTGCCTCTTTAGAGGGACATGCAGGAAGTTCT
GTTGATTTTGCTATTTTTTCTTTACATTTAGCTGGGGCTTCTTCTATTATAGGGGCAATTAATTTTATTTCCACTA
TTATTAATATGCGTGGATACGGTGTATCAATGGAAAAGGTTCCGTTATTTGTATGATCTGTTTTAATTACTGCG
GTTCTTTTGTTGTTATCATTACCTGTTTTAGCTGGTGCTATTACTATACTTTTAACTGATCGAAATTTTAATacttcg
ttttttgatcctgcaggaggaggagatcctgtgttatttcaa

>Tenuiphantes c.f. zimmermanni cytochrome oxidase subunit I gene, partial cds; mitochondrial
649bp
aactttatattttatttttggagcatgagctgcTATAGTAGGAACTGCTATAAGAGTGTTAATTCGAATTGAGTTGGGACAA
ACTGGAAGAATGTTAGGTGATGACCAGTTATATAATGTTATTGTTACTGCACATGCTTTTGTTATAATTTTTTTT
ATAGTTATGCCTATTTTAATTGGGGGATTCGGAAATTGGTTAGTGCCTTTAATACTTGGTGCTCCTGATATAGC
TTTTCCTCGAATAAATAATTTAAGATTTTGGCTTTTGCCTCCTTCTTTATTATTATTATTTATTTCAAGTATAGTTG
AAATAGGAGTTGGAGCAGGATGAACAGTGTATCCTCCTCTTGCTTCTTTAGAGGGGCATGCAGGAAGTTCTGT
TGATTTTGCTATTTTTTCTCTTCATTTAGCTGGAGCTTCTTCGATTATAGGAGCAATTAATTTTATCTCCACTATT
GTTAATATACGTGGATACGGTGTCACAATAGAAAAGGTTCCATTGTTTGTATGATCTGTTTTGATTACTGCTGT
TCTTTTATTGTTGTCGTTACCTGTTTTAGCAGGTGCTATTACTATACTTTTAACTGACCGCAATTTTAATACTTCG
TTTTTTGATcctgcgggtggaggggatcctgtattatttcaa

>Tenuiphantes tenuis cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
tactttatattttatttttggagcgtgagctgctatagtaggaactgctataagagtattGATTCGAATTGAGTTGGGTCAAACTGGG
AGAATATTGGGAGATGATCAGTTATATAATGTTATTGTCACTGCACATGCTTTTGTTATAATTTTTTTTATAGTT
ATACCTATTTTAATTGGGGGATTTGGAAATTGGTTAGTTCCTTTGATACTTGGGGCACCTGATATAGCTTTTCCT
CGAATAAATAATTTAAGCTTTTGACTTTTACCCCCTTCTTTGTTATTATTATTTATTTCAAGTATGGTTGAGATAG
GAGTTGGGGCAGGGTGAACAGTGTATCCTCCTCTTGCTTCTTTGGAAGGTCATGCAGGAAGTTCTGTGGACTT
TGCTATTTTTTCTCTTCATTTAGCTGGGGCTTCTTCTATTATAGGAGCAATTAATTTTATTTCTACTATTTTGAATA

TACGAGGGTATGGGGTATCTATAGAAAAAGTACCATTGTTTGTTTGATCTGTGTTAATTACTGCTGTTCTCTTA
TTATTATCATTACCTGTTTTAGCAGGTGCTATTACAATACTTTTAACTGAtcgaaattttaatacttcatttttgaccctgca
ggaggggagatccagtattattccaa

>Tenuiphantes tenuis cytochrome oxidase subunit I gene, partial cds; mitochondrial 649bp
tactttatattttatttttggagcgtgagctgcTATAGTAGGAACTGCTATAAGAGTATTGATTCGAATTGAGTTGGGTCAA
ACTGGGAGAATATTGGGAGATGATCAGTTATATAATGTTATTGTCACTGCACATGCTTTTGTTATAATTTTTTTT
ATAGTTATACCTATTTTAATTGGGGGATTTGGAAATTGGTTAGTTCCTTTGATACTTGGGGCACCTGATATAGC
TTTTCCTCGAATAAATAATTTAAGCTTTTGACTTTTACCCCCTTCTTTGTTATTATTATTTATTTCAAGTATGGTTG
AGATAGGAGTTGGGGCAGGGTGAACAGTGTATCCTCCTCTTGCTTCTTTGGAAGGTCATGCAGGAAGTTCTGT
GGACTTTGCTATTTTTTCTCTTCATTTAGCTGGGGCTTCTTCTATTATAGGAGCAATTAATTTTATTTCTACTATTT
TGAATATACGAGGGTATGGGGTATCTATAGAAAAAGTACCATTGTTTGTTTGATCTGTGTTAATTACTGCTGTT
CTCTTATTATTATCATTACCTGTTTTAGCAGGTGCTATTACAATACTTTTAACTGATCGAAATTTTAATACTTCAT
TTTTTgaccctgcaggaggggagatccagtattattccaa

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