This is an electronic appendix to the Biology Letter by Blaxter *et al.* 2004 DNA taxonomy of a neglected animal phylum: an unexpected diversity of tardigrades. *Proc. R. Soc. Lond.* B (Suppl.) **271**, S189–S192. (DOI 10.1098/rsbl.2003.0130.)

Electronic appendices are refereed with the text. However, no attempt has been made to impose a uniform editorial style on the electronic appendices.

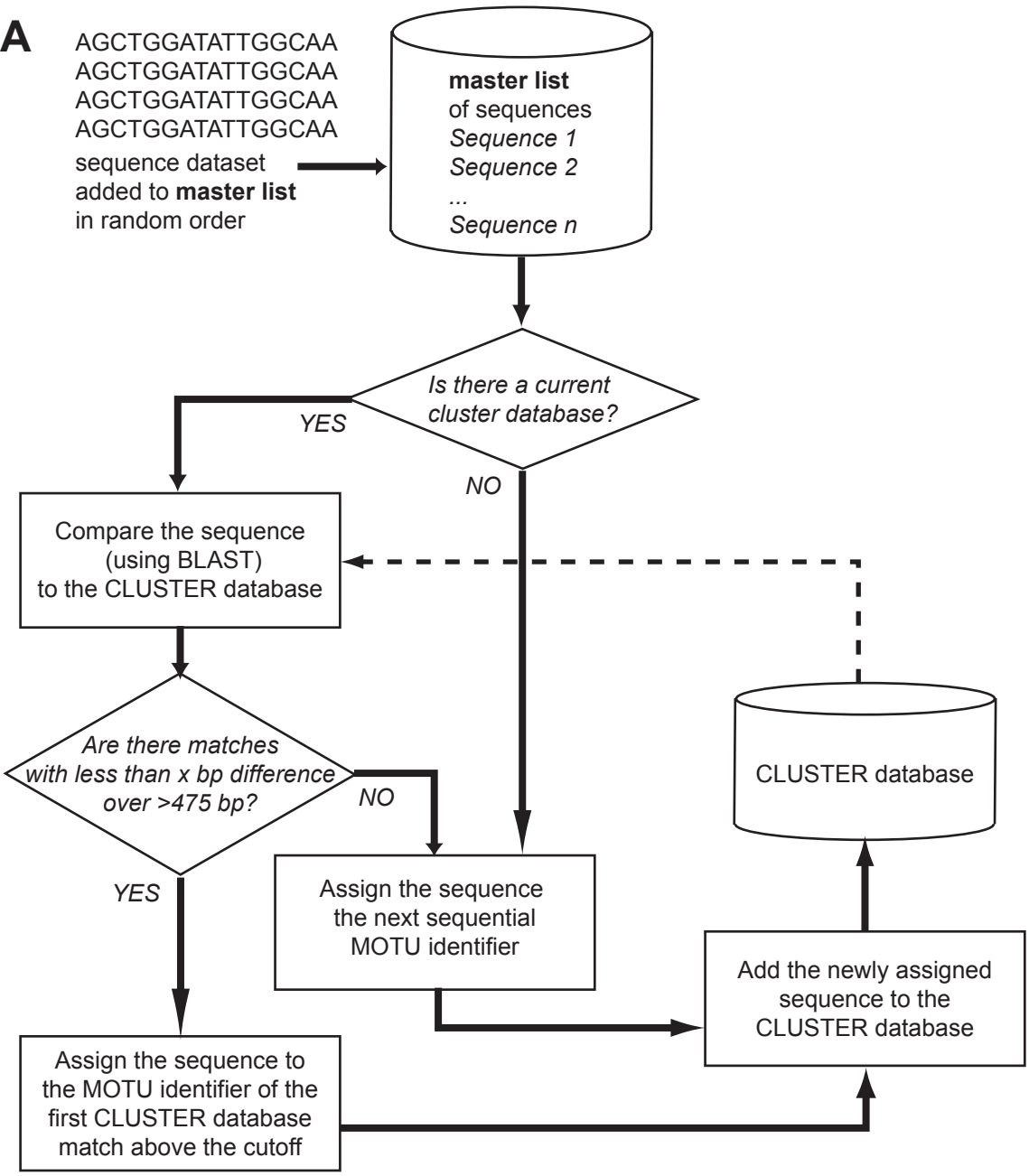
Electronic Appendix A

Figure 3: The process of molecular operational taxonomic unit assignment.

A: A flow diagram of the MOTU assignment process. Cylinders represent databases, diamonds decisions, and rectangles actions taken on the basis of decisions. The user can tell the process what level of discrimination ("x" base differences) is to be used. The process continues until all the sequences in the **master list** have been classified. The process can be rerun an indefinite number of times with randomisation of the order of sequences in the **master list**.

B: The MOTU process, because it deals with digital data, can result in varying MOTU assignment and numbers depending on sequence addition order. For a set of five example sequences **a** to **e**, the pairwise difference matrix shows that different numbers of MOTU will result from different addition orders. The results of twenty four such variations starting with sequence **a** are shown. This feature of the MOTU system is particularly evident in taxon "clouds" such as that of clade A in Figure 1.

A



B

sequence alignment (differences from **a** boxed)

```
a AGGAAATAGAGAACAGAGATACACAGATA
b AGGAAATAGAGAACAGAAATACACAGATA
c AGGAAAAAGAGAACAGAGATACACAGCTA
d AGGAAAATGAGAACAGAGATTCACAGCTA
e AGGAAAATGAGAACAGAAATACACAGATA
```

distance matrix

	b	c	d	e
a	1	2	4	3
b		3	5	2
c			2	3
d				3

addition order	number of 2bp MOTU
abcde,abced,abecd,acbed,acbde,acdbe	**1**
acdeb,acebd,acedb,adbce,adbec,aebcd,aecbd,aecdb,aedcb	**2**
abdce,abdec,abedc,adcbe,aedbc,adceb,adebc,adecb,aebdc	**3**