# Additional File 1

# Supplementary Information

Specimens whose gene sequences did not match published sequences with >95% identity, but whose closest alignments were to *Anopheles* species, were designated “unknown *Anopheles* species”. The information below provides a breakdown of the species that these sequences aligned to (on National Center for Biotechnology Information nucleotide database) at identity levels below that accepted to represent within-species variation.

COI sequences: unknown Anopheles species (n=163)

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| **Species category** | **Description** (incl. refs if appropriate) | | **Number of haplotypes (individuals);  proportion** |
| Sympatric species (<95% identity) | Anopheline species known to occur in the Afrotropical region | *An. species 1* [1, 2]*;  An. species 7* [3];  *An. coustani* group   (see designation in text); *An. gambiae* s.l.;  *An. squamosus* | 21 (22); 13% |
| Allopatric species (<95% identity) | Anopheline species *not* known  to occur in Afrotropical region | | 30 (114); 70% |
| Allopatric and sympatric species | Equal similarity (<95%) to both species categories | | 20 (27); 17% |

ITS2 sequences: unknown *Anopheles* species (n=52)

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| **Species category** | **Description** (incl. refs if appropriate) | | **Number individuals;  proportion** |
| Sympatric species (>95% identity) | Above-threshold match to anopheline species known to occur in Afrotropical region, without COI sequence | *An. pharoensis;*  *An. species 16* [3] | 3; 6% |
| Sympatric species (90-95% identity) | Anopheline species known to occur in the Afrotropical region | *An. c.f. coustani 1* [4]; *An. pharoensis;*  *An. species 16* [3] | 10; 19% |
| Sympatric species (<90% identity) | Anopheline species known to occur in the Afrotropical region | *An. coustani* s.s*.;*  *An. mascarensis;*  *An. moucheti;*  *An. species 6* [3];  *An. species 7* [3];  *An. species 9* [3];  *An. species 11* [3];  *An. theileri* | 37; 71% |
| Allopatric species (<95% identity) | Anopheline species *not* known  to occur in Afrotropical region | | 2; 4% |

References

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2. Lemma W, Alemu K, Birhanie M, Worku L, Niedbalski J, McDowell MA, et al. Anopheles cinereus implicated as a vector of malaria transmission in the highlands of north-west Ethiopia. Parasit Vectors.2019;12 1:557; doi: 10.1186/s13071-019-3797-9. <https://www.ncbi.nlm.nih.gov/pubmed/31767025>.

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4. Lobo NF, St Laurent B, Sikaala CH, Hamainza B, Chanda J, Chinula D, et al. Unexpected diversity of Anopheles species in Eastern Zambia: implications for evaluating vector behavior and interventions using molecular tools. Sci Rep-Uk.2015;5; doi: 10.1038/srep17952.