# 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.