

## Additional File 1

**Table S1: Unknown *Anopheles* species: cytochrome *c* oxidase 1 (COI) sequences.** Specimens whose COI sequences did not match published sequences with  $\geq 95\%$  identity (on National Center for Biotechnology Information nucleotide database), but whose closest alignments were to *Anopheles* species, were designated “unknown *Anopheles* species”. This table provides a breakdown of the species that these sequences aligned with at identity levels below that accepted to represent within-species variation.

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 <i>An. species 1</i> [1, 2]; <i>An. species 7</i> [3]; <i>An. coustani</i> group (see designation in text); <i>An. gambiae</i> s.l.; <i>An. squamosus</i>	21 (22); 13%
Allopatric species (<95% identity)	Anopheline species <i>not</i> known to occur in Afrotropical region	30 (114); 70%
Allopatric and sympatric species	Equal similarity (<95%) to both species categories	20 (27); 17%

**Table S2: Unknown *Anopheles* species: second Internal Transcribed Spacer (ITS2) region sequences.** Specimens whose ITS2 sequences did not match published sequences with  $\geq 95\%$  identity (on National Center for Biotechnology Information nucleotide database), but whose closest alignments were to *Anopheles* species, were designated “unknown *Anopheles* species”. This table provides a breakdown of the species that these sequences aligned with at identity levels below that accepted to represent within-species variation.

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	<i>An. pharoensis</i> ; <i>An. species 16</i> [3]	3; 6%
Sympatric species (90-95% identity)	Anopheline species known to occur in the Afrotropical region	<i>An. c.f. coustani</i> 1 [4]; <i>An. pharoensis</i> ; <i>An. species 16</i> [3]	10; 19%
Sympatric species (<90% identity)	Anopheline species known to occur in the Afrotropical region	<i>An. coustani</i> s.s.; <i>An. mascarensis</i> ; <i>An. moucheti</i> ; <i>An. species 6</i> [3]; <i>An. species 7</i> [3]; <i>An. species 9</i> [3]; <i>An. species 11</i> [3]; <i>An. theileri</i>	37; 71%
Allopatric species (<95% identity)	Anopheline species <i>not</i> 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;12:557; doi: 10.1186/s13071-019-3797-9.
3. St Laurent B, Cooke M, Krishnankutty SM, Asih P, Mueller JD, Kahindi S, et al. Molecular characterization reveals diverse and unknown malaria vectors in the western Kenyan highlands. *Am J Trop Med Hyg.* 2016;94:327-35; doi: 10.4269/ajtmh.15-0562.
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.