

1 Supplemental File 1 - Declaration of a new *Caenorhabditis* species

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11 *Caenorhabditis oiwi* Crombie et al. sp. n.

12 We isolated and identified a new *Caenorhabditis* species that we named *Caenorhabditis oiwi* sp. n. for the
13 Hawaiian word meaning native. Here, we justify the species status of *C. oiwi* sp. n. based on molecular
14 barcodes and biological species inference from mating experiments. The type isolate for *C. oiwi* sp. n. is
15 strain ECA821. We also made an isogenized version of ECA821 by ten generations of sib mating (named
16 ECA1100). The species reproduces sexually with males and females. The ITS2 sequence from ECA1100
17 *C. oiwi* sp. n. (Genbank Accession: MN056420) differs from that of all previously described *Caenorhabditis*
18 species for which such information is available (Félix et al., 2014; Huang et al., 2014; Kiontke et al., 2011;
19 Stevens et al., 2019). Note that these ribosomal DNA sequences might vary slightly within the species. Based
20 on molecular data, *C. oiwi* sp. n. falls into the Elegans supergroup of *Caenorhabditis* (Kiontke et al., 2011)
21 with the closest known species being *C. kamaaina* (Félix et al., 2014). Reciprocal mating experiments of
22 *C. oiwi* sp. n. ECA821 with the *C. kamaaina* type isolate QG122 did not yield any viable progeny. *C.*
23 *kamaaina* was previously described as a sister species to the *Japonica* group but was recently placed as the
24 most basally diverging species in the Elegans group (Kiontke et al., 2011; Stevens et al., 2018). The
25 discovery of *C. oiwi* sp. n. might help with resolving the shifting topology in this part of the *Caenorhabditis*
26 phylogenetic tree.

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28 The type isolate ECA821 was collected in August of 2017 from the Island of Oahu, Hawaii (21.33611°N, -
29 157.7999°W) where it was isolated from a cluster of freshly fallen flowers. ECA821 is deposited as a cryo-
30 preserved living stock at the *Caenorhabditis* Genetics Center. Isolate ECA821 is deposited in the NYU
31 Rhabditid Collection and was used to study the morphology of the species (**Supplemental Figure 12**;
32 **Supplemental Figure 13**). In agreement with the similarity of their rRNA sequences, *C. oiwi* sp. n. and *C.*
33 *kamaaina* are at present morphologically indistinguishable. Both species show the common features of the
34 Elegans group of *Caenorhabditis* (Sudhaus and Kiontke, 2007). Their lips are separate; the stoma is long
35 and bears three flaps of moderate size at the metastegostom (**Supplemental Figure 12A-B**). The male tail
36 shows the typical heart-shaped, anteriorly closed fan (bursa) with a serrated edge and a shallow terminal
37 notch (**Supplemental Figure 13A, E**). The nine pairs of rays are arranged as is typical for the Elegans group
38 with two pairs of rays positioned precloacally and the tips of ray pairs v1 are attached to the dorsal side of
39 the fan. The anterior dorsal ray (ad) is in position five and the posterior dorsal ray (pd) in position seven. The
40 tips of the sixth pair of rays (v5) are embedded in the cuticle. Rays v4 are much thinner and always shorter
41 than ad, a character that distinguishes *C. oiwi* sp. n. and *C. kamaaina* from most species of the Elegans
42 group (but not all; *C. doughertii*, *C. tropicalis* and *C. nigoni* also have a narrower and shorter ray v4). Several
43 species of the Japonica group show modified rays v4. In *C. japonica*, *C. nouraguensis*, *C. panamaensis* and
44 *C. waitukubuli*, rays v4 are much shorter than the ad rays. In *C. becei* and *C. macrosperma*, rays v4 are only
45 slightly shorter than the ad rays, but not as skinny as in *C. kamaaina* and *C. oiwi*. The spicules are slender
46 and their tip is pointed. The gubernaculum shows the usual forked distal tip and lateral ears (**Supplemental**
47 **Figure 13C, D**), but both are more prominent than in most other species of the Elegans group. Here, only *C.*
48 *inopinata* and *C. brenneri* have equally solid lateral ears and distal forked tip. The morphology of the females

49 (**Supplemental Figure 12B-F**) is in agreement with that of the stem species pattern of the Elegans group
50 (Sudhaus and Kiontke, 2007).