

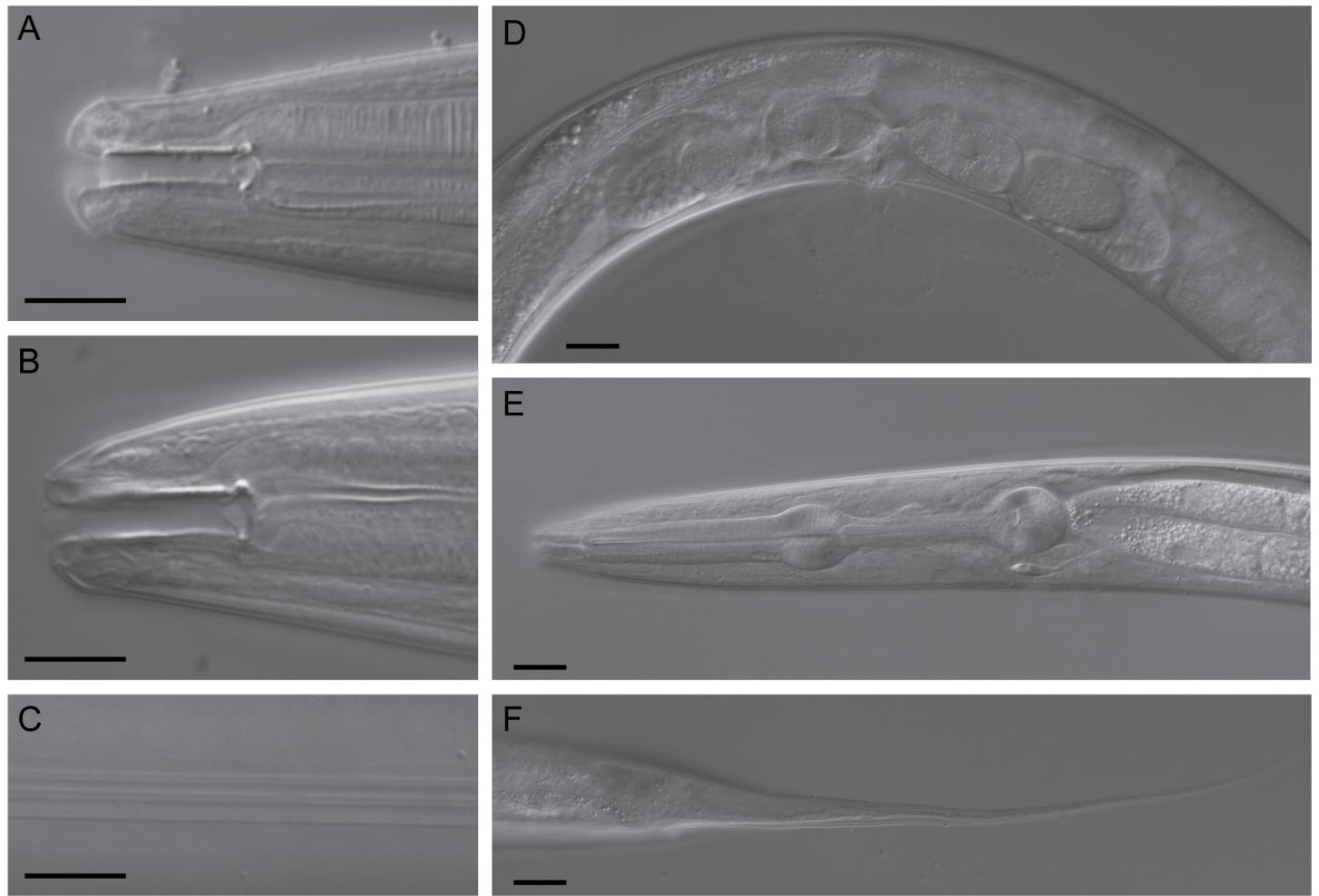
Supplemental File 1 - Declaration of a new *Caenorhabditis* species

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Caenorhabditis oiwi Crombie et al. sp. n.

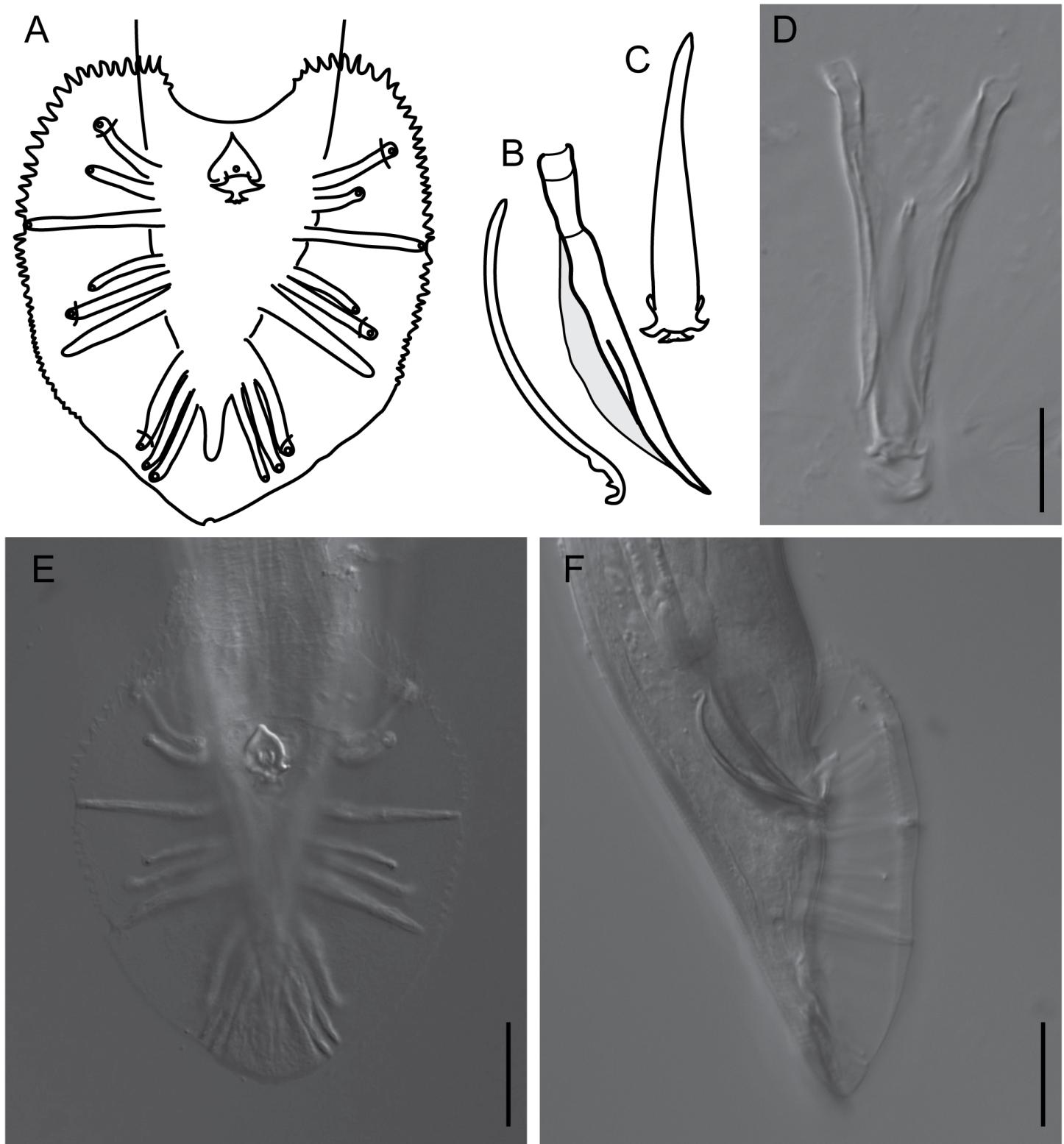
We isolated and identified a new *Caenorhabditis* species that we named *Caenorhabditis oiwi* sp. n. for the Hawaiian word meaning native. Here, we justify the species status of *C. oiwi* sp. n. based on molecular barcodes and biological species inference from mating experiments. The type isolate for *C. oiwi* sp. n. is strain ECA821. We also made an isogenized version of ECA821 by ten generations of sib mating (named ECA1100). The species reproduces sexually with males and females. The ITS2 sequence from ECA1100 *C. oiwi* sp. n. (Genbank Accession: [MN056420](#)) differs from that of all previously described *Caenorhabditis* species for which such information is available (Félix et al., 2014; Ferrari et al., 2017; Huang et al., 2014; Kiontke et al., 2011; Slos et al., 2017; Stevens et al., 2019). Note that these ribosomal DNA sequences might vary slightly within the species. Based on molecular data, *C. oiwi* sp. n. falls into the *Elegans* supergroup of *Caenorhabditis* (Kiontke et al., 2011) with the closest known species being *C. kamaaina* (Félix et al., 2014). Reciprocal mating experiments of *C. oiwi* sp. n. ECA821 with the *C. kamaaina* type isolate QG122 did not yield any viable progeny. *C. kamaaina* was previously described as a sister species to the *Japonica* group but was recently placed as the most basally diverging species in the *Elegans* group (Kiontke et al., 2011; Stevens et al., 2018). The discovery of *C. oiwi* sp. n. might help with resolving the shifting topology in this part of the *Caenorhabditis* phylogenetic tree.

The type isolate ECA821 was collected in August of 2017 from the Island of Oahu, Hawaii (21.33611°N, -157.7999°W) where it was isolated from a cluster of freshly fallen flowers. ECA821 is deposited as a cryopreserved living stock at the *Caenorhabditis* Genetics Center. Isolate ECA821 is deposited in the NYU Rhabditid Collection and was used to study the morphology of the species (**Supplemental Figures 13 and 14**). In agreement with the similarity of their rRNA sequences, *C. oiwi* sp. n. and *C. kamaaina* are at present morphologically indistinguishable. Both species show the common features of the *Elegans* group of *Caenorhabditis* (Sudhaus and Kiontke, 2007). Their lips are separate; the stoma is long and bears three flaps of moderate size at the metastegostom (**Supplemental Figure 13A-B**). The male tail shows the typical heart-shaped, anteriorly closed fan (bursa) with a serrated edge and a shallow terminal notch (**Supplemental Figure 14A, E**). The nine pairs of rays are arranged as is typical for the *Elegans* group with two pairs of rays positioned precloacally and the tips of ray pairs v1 are attached to the dorsal side of the fan. The anterior dorsal ray (ad) is in position five and the posterior dorsal ray (pd) in position seven. The tips of the sixth pair of rays (v5) are embedded in the cuticle. Rays v4 are much thinner and always shorter than ad, a character that distinguishes *C. oiwi* sp. n. and *C. kamaaina* from most species of the *Elegans* group (but not all; *C. doughertyi*, *C. tropicalis* and *C. nigoni* also have a narrower and shorter ray v4). Several species of the *Japonica* group show modified rays v4. In *C. japonica*, *C. nouraguensis*, *C. panamensis* and *C. waitukubuli*, rays v4 are much shorter than the ad rays. In *C. becei* and *C. macrosperma*, rays v4 are only slightly shorter than the ad rays, but not as skinny as in *C. kamaaina* and *C. oiwi*. The spicules are slender and their tip is pointed. The gubernaculum shows the usual forked distal tip and lateral ears (**Supplemental Figure 14C, D**), but both are more prominent than in most other species of the *Elegans* group. Here, only *C. inopinata* and *C. brenneri* have equally solid lateral ears and distal forked tip. The morphology of the females (**Supplemental Figure 13B-F**) is in agreement with that of the stem species pattern of the *Elegans* group (Sudhaus and Kiontke, 2007).



Supplemental Figure 14 - DIC micrographs of *C. oiwi* sp. n.

(A) stoma of male (subventral right, dorsal is up); (B) stoma of female subventral right, dorsal is up); (C) female lateral field with alae; (D) female midbody region showing vulva, one embryo in each uterus, one oocyte in each spermatheca and part of the posterior ovary (left side view); (E) pharynx region of female (left side view); and (F) female tail (left side view). Scale bars in A-C are 10 μ m and 20 μ m in D-F.



Supplemental Figure 14 - Features of the male tail of *C. oowi* sp. n.

(A) Drawing of the male tail in ventral view. The rays in position 1, 5 and 7 (from anterior) open to the dorsal side of the fan. (B) A drawing of the spicule and guernaculum in right lateral view is shown. (C) A drawing of the gubernaculum in ventral view is shown. (D) DIC micrograph of the spicules and gubernaculum in ventral view is shown. (E, F) DIC micrographs of the male tail in ventral (E) and lateral right view (F) are shown. Scale bars are 20 μ m.