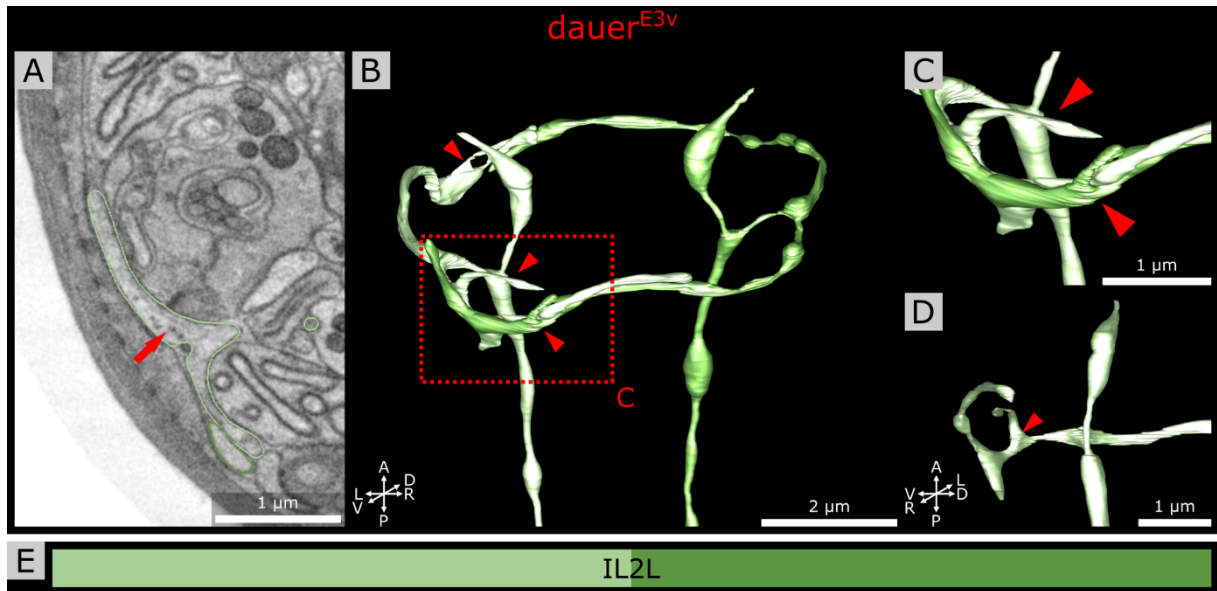


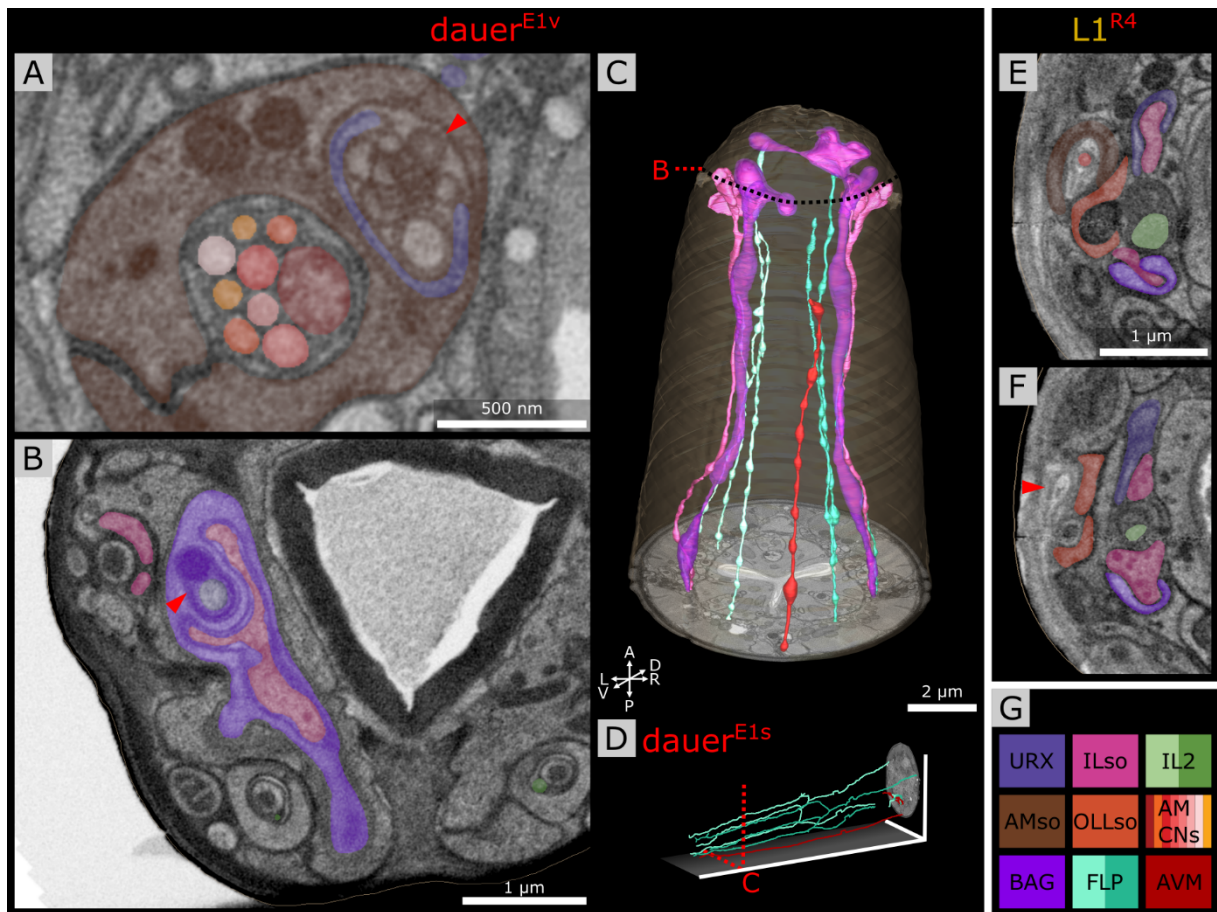
1

2 **Figure S1.** Anatomical features of the investigated dauer larvae and L1 larva. **(A)** Longitudinal virtual  
 3 section through the volumetric reconstructed image stack of the first dauer larva. The mouth is closed  
 4 (arrowhead). **(B)** Transverse section of the anterior worm tip showing dauer-specific cuticle fibers  
 5 (arrow). The nose appears flat as the six lips are not clearly constricted compared to the L1 larva in **K**.  
 6 **(C)** Transverse section in the mid-region of the worm showing reduced intestinal structures (dashed  
 7 outline) and alae (asterisk) in shape specific for dauer larvae. **(D-F)** Second dauer shown in the same  
 8 manner as the first dauer larva in **A-C**. **(G-I)** Third dauer shown in the same manner as the first dauer  
 9 larva in **A-C**. **(G,H)** The anterior tip is partly missing. **(J-L)** L1 larva shown in the same manner as the  
 10 first dauer larva in **A-C**. Note that the mouth is open (**J**, non-filled arrowhead). The six lips are more  
 11 prominently present, here visible for one lip curved on the side facing the mouth opening (**K**, arrowhead),  
 12 the intestinal structures are more expanded (**L**, dashed outline), and the alae of the L1 larva (**L**, asterisk)  
 13 differ in shape from those of dauer. **(M)** Alae of the L1 larva higher magnified. Note that there are two  
 14 cuticles. The alae have one big ridge arising from the upper cuticle. **(N)** SEM image of longitudinal  
 15 ultramicrotome section of the L1 larva focusing on the cuticle. The worm started to produce the next  
 16 cuticle. **(O)** Detail of **N**.



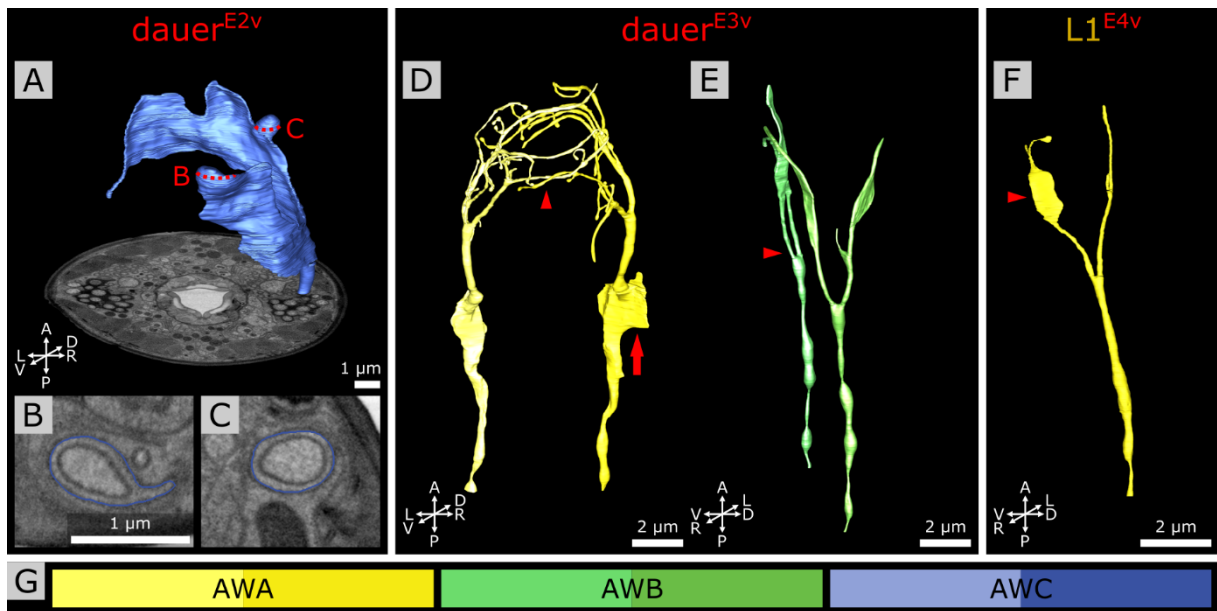
17

18 **Figure S2.** *Crown-like dendrites* of IL2L neurons in another dauer data set showing individual  
 19 differences. **(A)** Transverse FIB-SEM sections through the 3<sup>o</sup> dendrite of IL2LL of *Dauer*<sup>E3v</sup>. A few  
 20 electron dense vesicular structures are visible in the *crown-like dendrite* (arrow). **(B)** Volumetric  
 21 reconstruction of the anterior IL2L neuron endings of *Dauer*<sup>E3v</sup>. Crown-like dendrites of both IL2L  
 22 neurons extend 4<sup>o</sup> dendrites (arrowheads). **(C)** 4<sup>o</sup> dendrites shown in **B** higher magnified. **(D)** One 4<sup>o</sup>  
 23 dendrite (arrowhead) of IL2LL from another perspective. **(E)** Names and color code of cells shown.



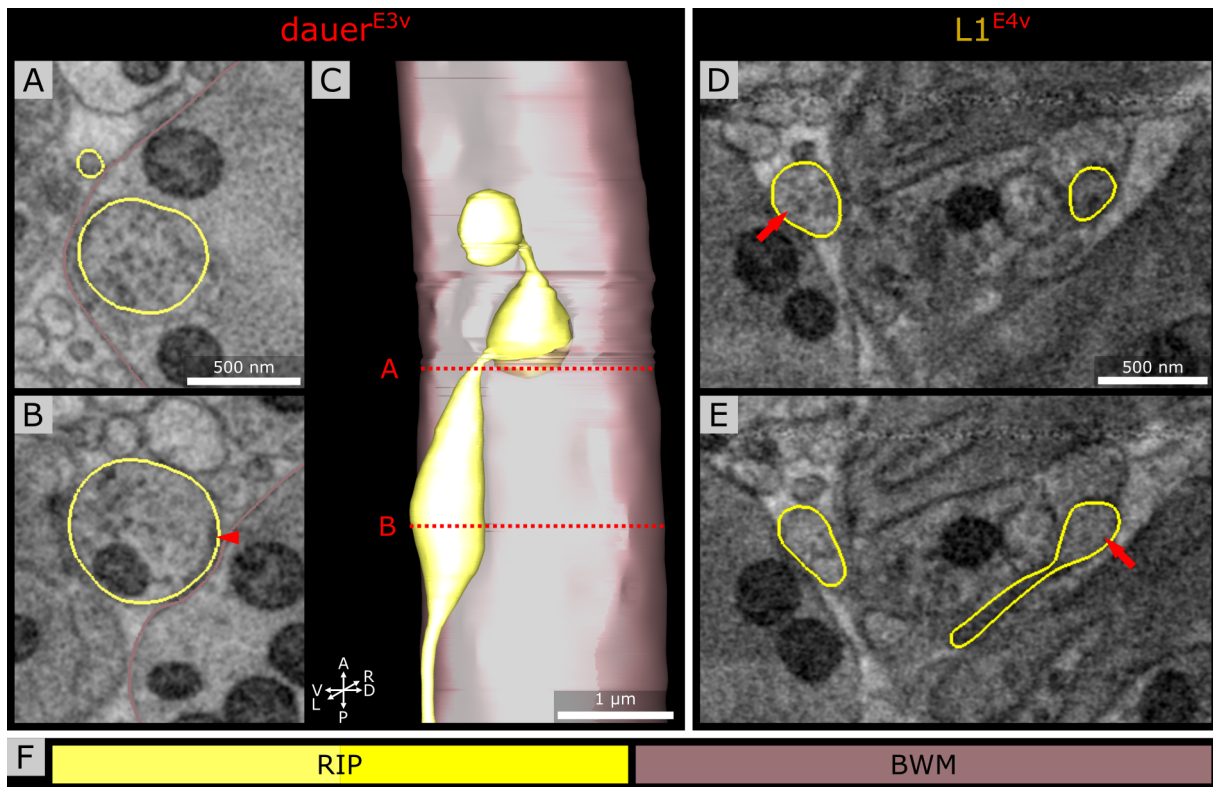
24

25 **Figure S3.** Further investigation of ILLso and AMso cell endings as well as non-sensilla neurons. (A)  
 26 URXL is enclosed by AMsoL cell and encloses big vesicular structures (arrowhead). (B) BAGL ending  
 27 encloses one branch of ILLsoL. BAGL also encloses two vesicular structures (arrowhead) of which one  
 28 is electron dense and the other might originate from URXL. (C) Volumetric 3D reconstruction of ILsoL  
 29 cells with two branches each of which one is enclosed by BAG endings while FLP do not have any  
 30 branch or ILso-interaction. AVM reaches almost into the anterior sensilla region. (D) Tracing of FLP  
 31 neurons and the AVM neuron. (E) Transverse section of ILLsoL ending in an L1 larva. ILLsoL has two  
 32 branches which are enclosed by URXL and BAGL. URXL is not enclosed by AMsoL cell. (F) OLLsoL  
 33 cell encloses the amphid opening which is in this case formed by respective Hyp cell (arrowhead). (G)  
 34 Names and color code of shown cells.



35

36 **Figure S4.** Individual findings of amphid wing neuron endings in dauer larvae and one L1 larva. **(A)**  
 37 Volumetric reconstruction of the AWCR ending of *Dauer<sup>E2v</sup>* fusing twice with itself (dashed lines) to a  
 38 capsule shape. **(B,C)** Transverse sections through the AWCL capsules from **A**. **(D)** Volumetric  
 39 reconstruction of AWA neuron endings of *Dauer<sup>E3v</sup>* which are widely intertwining (arrowhead). AWAR  
 40 shows a wing-like thickening at the cilia base (arrow). **(E)** Volumetric reconstruction of AWB neuron  
 41 endings. The two AWBL branches both project into the ventral side (arrowhead). **(F)** Volumetric  
 42 reconstruction of AWAR neuron ending of *L1<sup>E4v</sup>* which has a wing-like morphology at one branch  
 43 (arrowhead). **(G)** Names and color code of shown cells.



44

45 **Figure S5.** RIP anterior endings enter the pharyngeal nervous system in another dauer individual and  
 46 in one L1 larva. **(A)** Section of the RIPL ending enclosed by BWM cell and filled with electron dense  
 47 vesicles. **(B)** Section of RIPL ending showing an electron dense projection facing the respective BWM  
 48 cell (arrowhead). **(C)** Volumetric reconstruction of the RIPL ending. RIPL has a big bouton enclosed by  
 49 respective BWM cell (section in **A**) before its ending is entering the pharynx. The ending inside the  
 50 pharyngeal nervous system has a bouton-like structure as well. **(D)** Section of RIPR ending of an L1  
 51 larva showing a bouton with electron dense vesicles (arrow) before entering the pharyngeal nervous  
 52 system like in dauer but to a lesser extent. **(E)** Section of RIPR ending inside the pharyngeal nervous  
 53 system just passing the pharyngeal basal lamina with a bouton-like structure (arrow) but again to a  
 54 lesser extent compared to dauer.

- 55 **Video S1.** Volumetric reconstruction of the cell endings at the anterior tip of a *C. elegans* dauer larva.
- 56 See Supplementary Material.
- 57
- 58 **Video S2.** Dendritic branches of the IL2Q neurons in one dauer individual in the context of the complete  
59 image stack and volumetric reconstruction of the anterior worm cell endings.
- 60 See Supplementary Material.

61 **Table S1.** Stage specific anatomical features for identification of dauer and L1 stage under starving  
 62 conditions.

Determination	Existence or shape of feature	Data set	
		E1-3	E4
Stage	<b>Cuticle</b>	Not investigated.	Next cuticle layer formation in process, indicating active development (Popham and Webster, 1979) and the analyzed larva stage to be just before its end. Excluding developmental arrest. See <b>Supplementary Figure S1M-O.</b>
	<b>Alae</b>	Existing, like known dauer-specific shape (Cassada and Russell, 1975; Singh and Sulston, 1978; Cox et al., 1981; Albert and Riddle, 1983). See <b>Supplementary Figure S1C,F,I.</b>	Existing, alae on the outer cuticle layer similar to known L1-specific shape (Cox et al., 1981) and like shape with second cuticle (Popham and Webster, 1979). Excluding L2d-dauer molt as dauer-specific alae would be on the inner cuticle (Singh and Sulston, 1978). See <b>Supplementary Figure S1L,M.</b>
	<b>Mouth opening</b>	Closed, like known for dauer and L2d-dauer molt (Albert and Riddle, 1983; Golden and Riddle, 1984). See <b>Supplementary Figure S1A,D,G.</b>	Open, known for L1 (Baugh and Sternberg, 2006; Baugh et al., 2009) and excluding L2d-dauer molt as it would be closed (Golden and Riddle, 1984). See <b>Supplementary Figure S1J.</b>
	<b>Lips</b>	Less distinct, like known for dauer which let the <i>C. elegans</i> nose appear flatter than that of L2 stages (Albert and Riddle, 1983). Not clearly observable in data set E3 as structure is partly broken. See <b>Supplementary Figure S1B,E,H.</b>	More prominent lips than the other larvae. See <b>Supplementary Figure S1K.</b>
	<b>Cuticular fibers</b>	Existing, like known for dauer around their mouth opening (Albert and Riddle, 1983). Not observable in data set E3 as structure is partly broken. See <b>Supplementary Figure S1B,E,H.</b>	Not existing, excluding dauer as it was only observed in dauers up to now (Albert and Riddle, 1983). See <b>Supplementary Figure S1K.</b>
	<b>Intestine</b>	Reduced, like known for dauer (Popham and Webster, 1979). See <b>Supplementary Figure S1C,F,I.</b>	Not reduced, similar to known shape of L1 larva (Popham and Webster, 1979; Maduro, 2017). See <b>Supplementary Figure S1L.</b>

63

...

64

Determination	Existence or shape of feature	Data set	
		E1-3	E4
Stage	Length	435 $\mu\text{m}$ measured for E3, similar known for dauer (Cassada and Russell, 1975; Cox et al., 1981). Measurement only possible for E3.	Measurement not possible.
	Diameter	~14-18 $\mu\text{m}$ measured in the body middle segment, similar known for dauer (Cassada and Russell, 1975; Cox et al., 1981).	~17 $\mu\text{m}$ measured in the body middle segment, similar known for L1 (Cassada and Russell, 1975; Cox et al., 1981).
Biological sex	CEM neurons	Not existing, excluding male as it is male-specific (Sulston et al., 1983).	Not existing, excluding male as it is male-specific (Sulston et al., 1983).
Result		<b>Stage: Dauers</b> <b>Sex: Hermaphrodites</b>	<b>Stage: L1</b> <b>Sex: Hermaphrodite</b>



67 **Table S2.** Properties of acquired FIB-SEM data sets.

<b>Data set</b>	<b>Image data</b>	<b>Body segment</b>	<b>Direction of acquisition</b>
<b><i>Dauer<sup>E1</sup></i></b>	82.312 $\mu\text{m}$ with 10,289 image slices. Voxel size: 5 x 5 x 8 nm. Note: data set uploaded to CATMAID contains six images more.	From the amphid commissure to the anterior worm end.	Transversal from posterior to anterior.
<b><i>Dauer<sup>E2</sup></i></b>	7.768 $\mu\text{m}$ with 971 image slices. Voxel size: 5 x 5 x 8 nm.	From the Z-level where microvilli of AFD neurons are visible to the anterior worm end.	Transversal from posterior to anterior.
<b><i>Dauer<sup>E3</sup></i></b>	18.4 $\mu\text{m}$ with 2,300 image slices. Voxel size: 5 x 5 x 8 nm.	From the Z-level posterior to where the amphid neurons just are getting enclosed by the AMsh cell to the anterior worm end.	Transversal from posterior to anterior.
<b><i>L1<sup>E4</sup></i></b>	18.770 $\mu\text{m}$ with 3,754 image slices after stack transformation. Voxel size: 5 x 5 x 5 nm.	Anterior worm end.	Longitudinal from ventral to dorsal.

## 69 **Figure and video creation**

70 All figures were arranged with the software Inkscape (Inkscape.org Team, 2021). Schemes  
71 were created as vector graphics with Inkscape on basis of pixel snapshots from contour  
72 drawings from 3dmod (Kremer et al., 1996) reconstruction or vector graphic snapshots from  
73 skeleton tracing from CATMAID (Saalfeld et al., 2009). 2D labels from 3dmod were merged  
74 with respective EM images in GIMP as separated 3dmod pixel snapshots (GIMP.org Team,  
75 2021). Brightness and contrast of EM images in 3D view of 3dmod were post-adjusted with  
76 3dmod. Snapshots of **Supplementary Figure S1D,G** were post-denoised with VSNR plugin in  
77 Fiji and once more brightness and contrast re-adjusted in 3dmod. 3dmod was also used to adjust  
78 images shown in 3D view of CATMAID. After adjustment, images were then merged with the  
79 tracing skeleton vector graphics in Inkscape. These EM images were also rendered manually  
80 by blacking pixels on the outside around the worm with the software GIMP. For **Figure 1B**,  
81 EM images were rendered by creating as mask from the outline of the body wall cuticle from  
82 3dmod and subtracting it from the image stack in Fiji (Schindelin et al., 2012). This rendered  
83 image stack was also taken for **Supplementary Videos S1, S2**. Image sequences for videos  
84 were created with 3dmod. Video labels were created with Inkscape. Labels and snapshots were  
85 merged with the Python Imaging Library Pillow (Python-Pillow.org Team, 2021) and exported  
86 as video with the OpenCV-Python library (opencv-python Team, 2021). For **Supplementary**  
87 **Video S2**, tracing skeletons from CATMAID were transformed into a 3dmod model. For that,  
88 coordinates of tracing nodes were exported as .csv file from the 3D view of CATMAID,  
89 prepared, and then imported into 3dmod with the *mod2point* function.

## 90 References Supplement

91

92 Albert, P. S., and Riddle, D. L. (1983). Developmental alterations in sensory neuroanatomy of  
93 the *Caenorhabditis elegans* dauer larva. *J. Comp. Neurol.* 219, 461–481.  
94 doi:10.1002/cne.902190407.

95 Baugh, L. R., DeModena, J., and Sternberg, P. W. (2009). RNA Pol II Accumulates at  
96 Promoters of Growth Genes During Developmental Arrest. *Science* 324, 92–94.  
97 doi:10.1126/science.1169628.

98 Baugh, L. R., and Sternberg, P. W. (2006). DAF-16/FOXO Regulates Transcription of cki-  
99 1/Cip/Kip and Repression of lin-4 during *C. elegans* L1 Arrest. *Curr. Biol.* 16, 780–785.  
100 doi:10.1016/j.cub.2006.03.021.

101 Cassada, R. C., and Russell, R. L. (1975). The dauerlarva, a post-embryonic developmental  
102 variant of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 46, 326–342.

103 Cox, G. N., Staprans, S., and Edgar, R. S. (1981). The cuticle of *Caenorhabditis elegans*: II.  
104 Stage-specific changes in ultrastructure and protein composition during postembryonic  
105 development. *Dev. Biol.* 86, 456–470. doi:10.1016/0012-1606(81)90204-9.

106 GIMP.org Team (2021). *GIMP Version 2.10.24*. Available at: <https://www.gimp.org/>  
107 [Accessed June 13, 2021].

108 Golden, J. W., and Riddle, D. L. (1984). The *Caenorhabditis elegans* dauer larva:  
109 Developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102, 368–378.  
110 doi:10.1016/0012-1606(84)90201-X.

111 Inkscape.org Team (2021). *Inkscape Version 1.1*. Available at: <https://inkscape.org/> [Accessed  
112 June 13, 2021].

113 Kremer, J. R., Mastronarde, D. N., and McIntosh, J. R. (1996). Computer visualization of three-  
114 dimensional image data using IMOD. *J. Struct. Biol.* 116, 71–76.  
115 doi:10.1006/jsbi.1996.0013.

116 Maduro, M. F. (2017). Gut development in *C. elegans*. *Semin. Cell Dev. Biol.* 66, 3–11.  
117 doi:10.1016/j.semcdb.2017.01.001.

118 opencv-python Team (2021). *opencv-python*. OpenCV Available at:  
119 <https://github.com/opencv/opencv-python> [Accessed June 13, 2021].

120 Popham, J. D., and Webster, J. M. (1979). Aspects of the fine structure of the dauer larva of the  
121 nematode *Caenorhabditis elegans*. *Can. J. Zool.* 57, 794–800. doi:10.1139/z79-098.

122 Python-Pillow.org Team (2021). *Python Pillow*. Available at: <https://python-pillow.org/>  
123 [Accessed June 13, 2021].

124 Saalfeld, S., Cardona, A., Hartenstein, V., and Tomančák, P. (2009). CATMAID: collaborative  
125 annotation toolkit for massive amounts of image data. *Bioinformatics* 25, 1984–1986.  
126 doi:10.1093/bioinformatics/btp266.

- 127 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al.  
128 (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9,  
129 676–682. doi:10.1038/nmeth.2019.
- 130 Singh, R. N., and Sulston, J. E. (1978). Some Observations On Moulting in *Caenorhabditis*  
131 *Elegans*. *Nematologica* 24, 63–71. doi:10.1163/187529278X00074.
- 132 Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell  
133 lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100, 64–119.  
134 doi:10.1016/0012-1606(83)90201-4.
- 135