

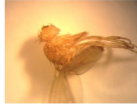
Supplementary Figure 1

Arthropods

Mollusk



Anisoptera
(Dragonfly)



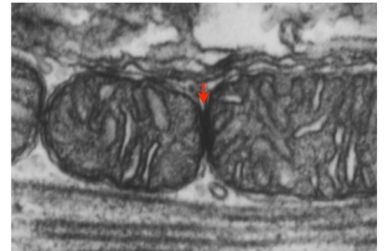
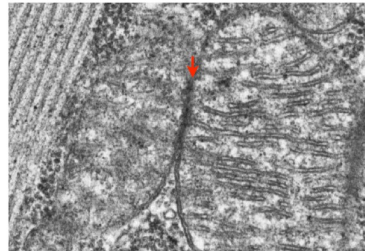
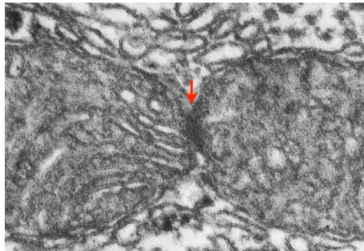
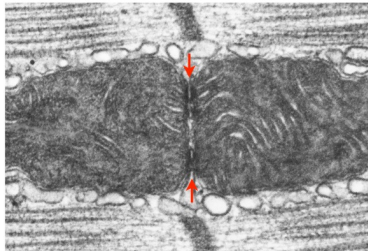
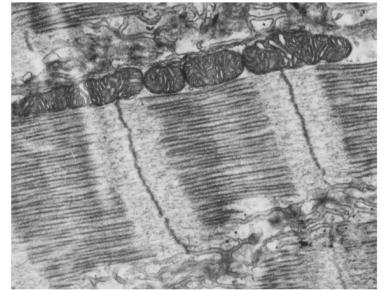
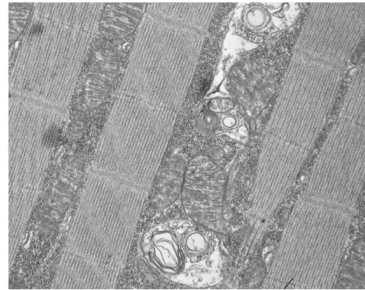
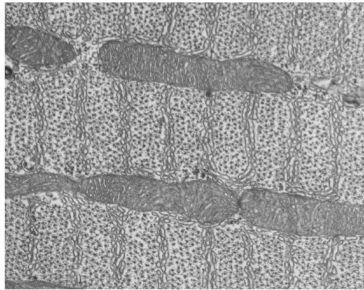
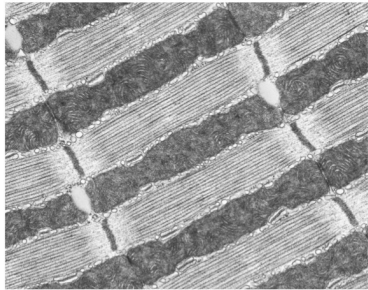
Drosophila
(Fruit fly)



Calliphora
(House fly)

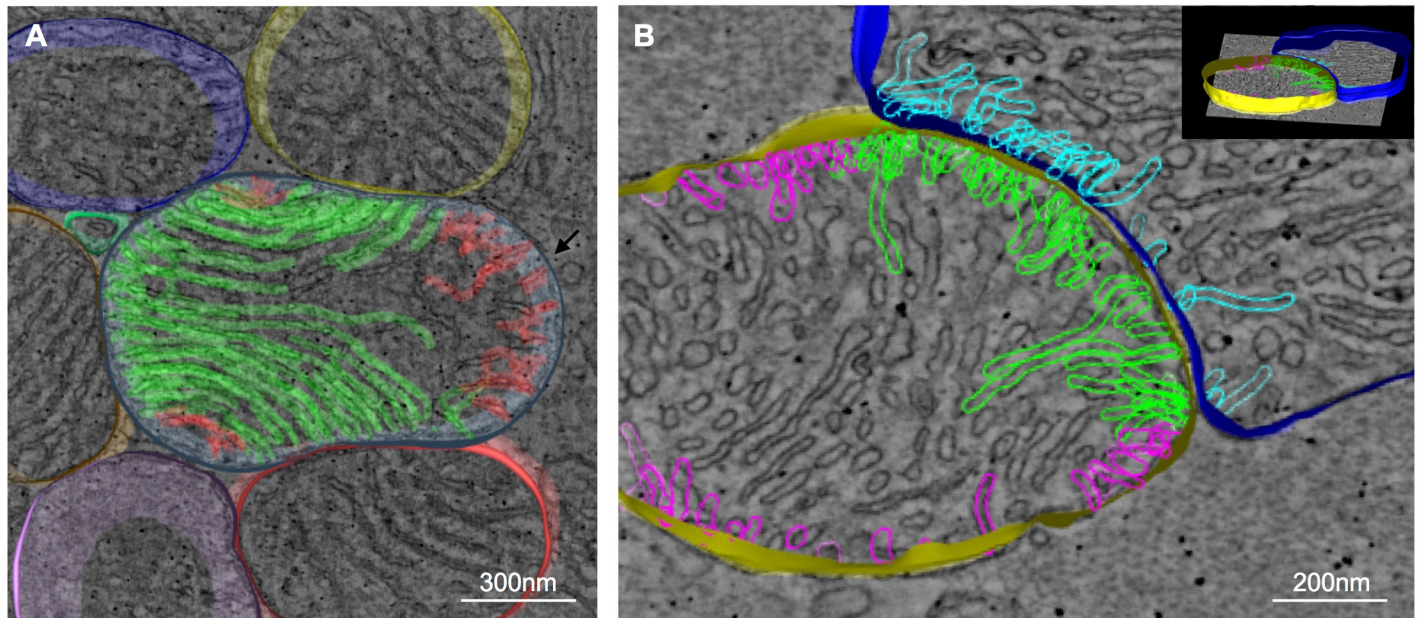


Pectinidae
(Scallop)



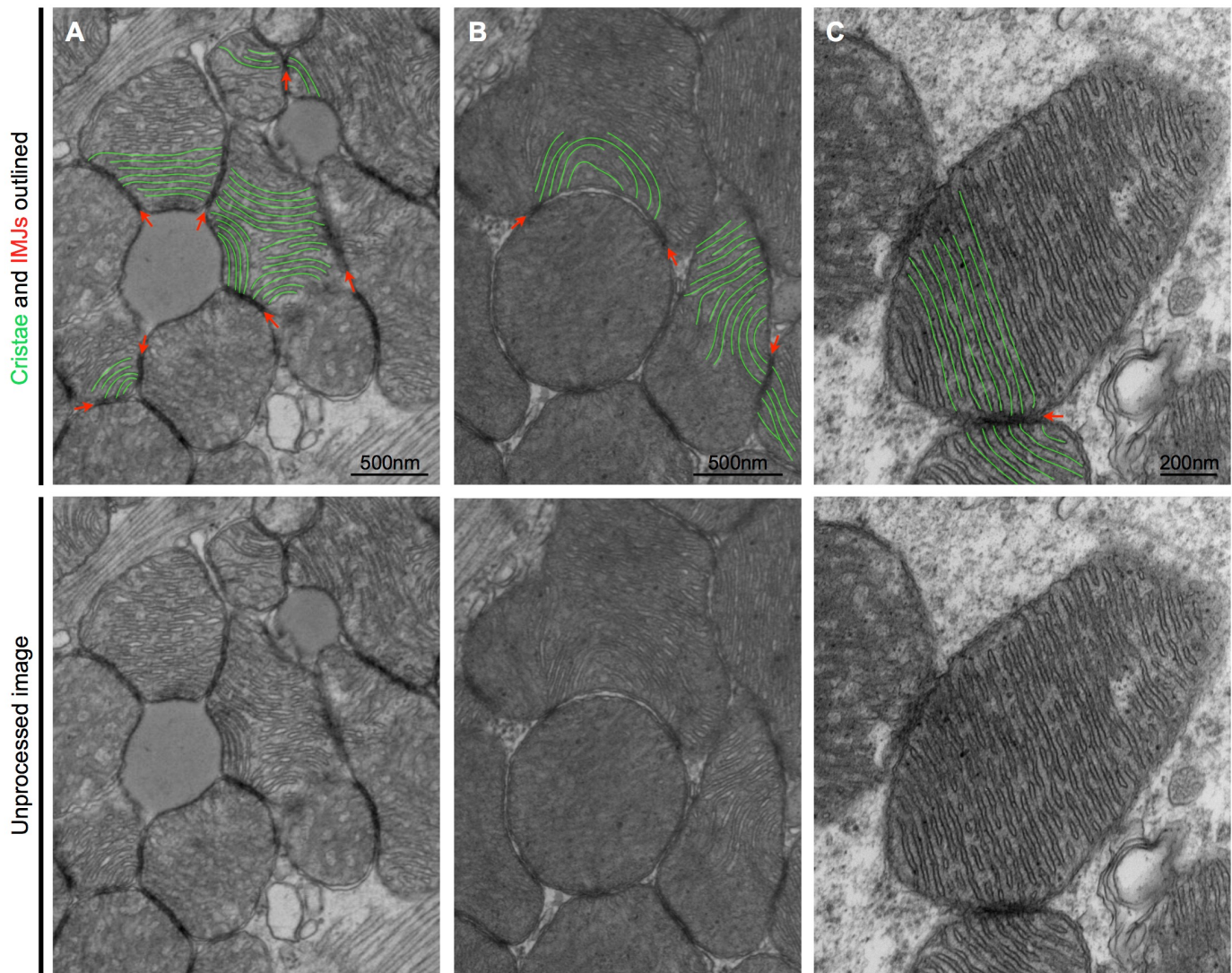
Electron-dense inter-mitochondrial junctions (IMJs) and associated cristae organization are evolutionary conserved features of mitochondria-mitochondria contacts. Shown are electron micrographs from three arthropod species and one mollusk muscle at low (top) and higher (bottom) magnification. Samples were fixed by immersion with glutaraldehyde, as described in the methods. Images were kindly provided by Dr. Clara Franzini-Armstrong.

Supplementary Figure 2



Electron microscopy tomographic reconstructions show cristae number, length and organization. (A) Electron tomogram (thickness = 197nm) of mouse heart cardiomyocyte mitochondria, generated as described in materials and methods. Mitochondrial membranes are outlined in colours. Profiles of uninterrupted cristae originating from IMJs (green) and non-IMJ membrane (red), showing both cristae density and length. Note the discontinuous and heterogenous orientation of cristae originating from areas of mitochondria facing myofibrils (arrow), compared to areas of the same central mitochondrion in contact with other mitochondria. **(B)** Electron tomogram (thickness: 157nm) of mouse skeletal muscle generated as in A to quantify the number of cristae junctions.

Supplementary Figure 3



Electron micrographs showing cristae organization and their relation to IMJs. (A, B) Mouse heart cardiomyocyte and **(C)** skeletal muscle (soleus) mitochondria, with selected cristae outlined in green. Shown are both raw images (bottom) and with cristae tracing outlined (top). Image in B kindly provided by Drs. Yan Burelle and Virgilio Cadete.

Supplementary Table 1

Species	Tissue	Cell type/sub-region	Reference
Mouse	Heart	Cardiomyocyte, left myocardium	1
	Muscle	Soleus	2, 3
Rat	Heart	Cardiomyocytes	4
	Heart	Cultured primary cardiomyocytes	5
	Diaphragm	Skeletal muscle fibers	6
Human	Eye	Retina	7
	Tumor	Glioma	8
Lamprey	Brain	Presynaptic terminal (tonic synapses > phasic synapses)	9
Bat	Fat	Brown adipose tissue	7
Squirrel	Brain	Hippocampus, CA1 neurons	10
Hamster	Adrenal gland	Cortex, zona fasciculata	7
Frog	Heart	Cardiomyocytes	11

Non-comprehensive list of published sources where inter-mitochondrial junctions (IMJs) can be observed.

Supplementary References

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