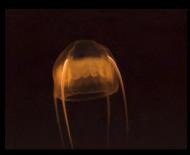




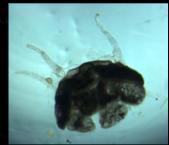


# The perils of bad taxonomy for leading edge science: a case study with the genus *Aegina*, and the consequences for Deep Learning









Dhugal Lindsay (JAMSTEC),

Mary Grossmann, Mitsuko Hidaka (JAMSTEC/Kitasato U.), Jun Nishikawa (Tokai U.), Hiroshi Miyake (Kitasato U.), Ryo Minemizu, Russell Hopcroft (U. Alaska), Bastian Bentlage (U. Guam), Allen Collins (Smithsonian), Takehisa Yamakita (JAMSTEC), Hiroyuki Yamamoto (JAMSTEC)

# Aegina citrea — Case Study Treated as a single species for the last >50 years



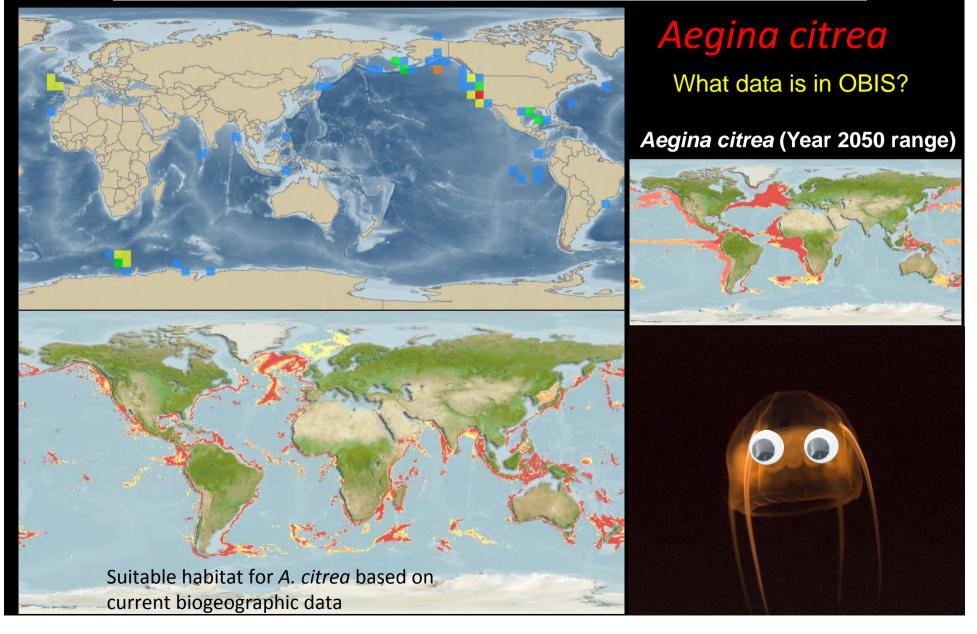
Narcomedusae Aeginidae

Kramp (1961) synonymized

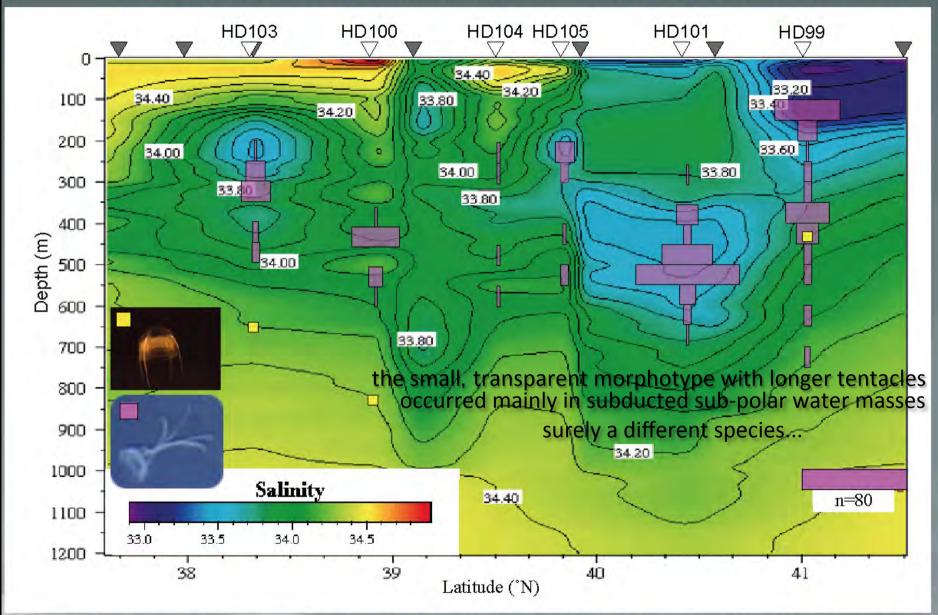
- 1 Aegina rosea Eschscholtz, 1829
- 2 Aegina rhodina Haeckel, 1879
- 3 Aegina eschscholtzii Haeckel, 1879
- 4 Aegina lactea Vanhöffen, 1908
- 5 Aegina brunnea Vanhöffen, 1908
- 6 Aegina alternans Bigelow, 1909
- 7 Aegina pentanema Kishinouye, 1910
- 8 Cunarcha aeginoides Haeckel, 1879

Basically, any narcomedusa with 4-6 tentacles, 8-12 stomach pouches and a peripheral canal system were all relegated to this one species

# Aegina citrea: A Poster Child for determining environmental niches and assessing the effects of climate change



### Only with Correct Taxonomy can Niches be characterised



Horizontal and vertical distribution of "Aegina citrea" over the Japan Trench from 20 April to 6 May 2002. Salinity profiles obtained using a Seabird SBE-19 CTD mounted on the ROV HYPER-DOLPHIN (white triangles) and X-CTD probes deployed between dive points (grey triangles).

## Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematical taxa

ALLEN G. COLLINS<sup>1</sup>, BASTIAN BENTLAGE<sup>2</sup>, ALBERTO LINDNER<sup>3</sup>, DHUGAL LINDSAY<sup>4</sup>, STEVEN H.D. HADDOCK<sup>5</sup>, GERHARD JARMS<sup>6</sup>, JON L. NORENBURG<sup>7</sup>, THOMAS JANKOWSKI<sup>8</sup> AND PAULYN CARTWRIGHT<sup>2</sup>

We pointed out in this 2008 paper that Aegina citrea and A. rosea were genetically different enough to be put in 2 separate families









#### However to our dismay...

Aegina rosea small subunit 18S ribosomal RNA gene, partial sequence

GenBank: EU247813.1



Aegina rosea small subunit 18S ribosomal RNA gene

**FASTA Graphics PopSet** 

Go to:

LOCUS EU247813 1739 bp DNA linear INV 05-MAY-2014

DEFINITION Aegina rosea small subunit 18S ribosomal RNA gene, partial sequence.

ACCESSION EU247813

VERSION EU247813.1 GI:166007992

KEYWORDS .

SOURCE Aegina citrea

ORGANISM Aegina citrea

Eukaryota; Metazoa; Cnidaria; Hydrozoa; Trachylina; Narcomedusae; Aeginidae; Aegina.

REFERENCE 1 (bases 1 to 1739)

AUTHORS Collins, A.G., Bentlage, B., Lindsay, D., Haddock, S.H.D., Lindner, A., Norenburg, J.L., Jarms, G., Jankowski, T. and Cartwright, P.

TITLE Phylogenetics of Trachylina (Cnidaria: Hydrozoa)

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 1739)

AUTHORS Collins, A.G., Bentlage, B., Lindsay, D., Haddock, S.H.D., Lindner, A., Norenburg, J.L., Jarms, G., Jankowski, T. and Cartwright, P.

TITLE Direct Submission

JOURNAL Submitted (25-OCT-2007) Dept. of Ecology and Evolutionary Biology,

The University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045, USA



somehow our *Aegina rosea* sequence has been labelled Aegina citrea, even though our paper described family level differences and we registered it as Aegina rosea!!!

**FEATURES** 

Location/Qualifiers

1..1739 source

> /organism="Aegina citrea" /mol\_type="genomic DNA"

/specimen voucher="JAMSTEC; dive, RB-MOC1-001

/db xref="taxon:168710"

<1..>1739 rRNA

/product="18S small subunit ribosomal RNA"



## Integrated Taxonomic Information System

Two main taxonomic databases that GenBank refers to for its own taxonomic database

Home

About

Welcome to ITIS, the Integrated Taxonomic Information System! Here you will find authoritative taxonomic information on plants, animals, fungi, and microbes of North America and the world. We are a partnership of U.S., Canadian, and Mexican agencies (ITIS-North America); other organizations; and taxonomic specialists. ITIS is also a partner of Species 2000 and the Global Biodiversity Information Facility (GBIF). The ITIS and Species 2000 Catalogue of Life (CoL) partnership is proud to provide the taxonomic backbone to the Encyclopedia of Life (EOL).



Home	WoRMS taxon details
About	Working taxon actums
Search taxa	✓ Aegina citrea Eschscholtz, 1829
Taxon tree	AphiaID: 117263
Literature	Classification: Biota > Animalia (Kingdom) > Cnidaria (Phylum) > Hydrozoa (Class) > Trachylinae (Subclass) > Narcomedusae (Order) > Aeginidae (Family) > Aeginia (Genus)
Distribution	
Specimens	Status accepted
Match taxa	Rank Species
Editors	Parent
Statistics	Synonymised Aegina rosea Eschscholtz, 1829 (Synonym)
Users	names
Webservice	Sources original description Eschscholtz, F. 1829. System der Acalephen. Eine ausführliche Beschreibung aller medusenartigen Strahltiere. Ferdinand Dümmler, Berlin, pp. 1-190, 116 pls., available online at http://www.biodiversitylibrary.org/item/40493#page/5/mode/1up
Photogallery	page(s): 113 (details)
Info downloads	[show all]
Sponsors	Vernacular Language Name
Glossary	Names English ? golf tee medusa [details]
Manual	Environment marina



Home

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Data Access and Tools

**Get ITIS Data** 

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#### Aegina Eschscholtz, 1829 Taxonomic Serial No.: 51145

#### in ITIS Aegina contains 3 valid species

Download data (Download Help) Aegina TSN 51145

#### Taxonomy and Nomenclature

Kingdom: Taxonomic Rank:

Genus

Synonym(s): Common Name(s):

#### **Taxonomic Status:**

Current Standing:

valid

#### **Data Quality Indicators:**

Record Credibility Rating: verified - standards met

Global Species Completeness: partial Latest Record Review: 2006

#### Taxonomic Hierarchy

Animalia - Animal, animaux, animals Kingdom

Subkingdom

Cnidaria Hatschek, 1888 - cnidarians, coelenterates, cnidaires, coelentérés, água viva, anêmona, caravela, cnidario, Phylum

coral, hidra

Subphylum Medusozoa Class

Hydrozoa Owen, 1843 - hydralike animals, hydroids, hydrozoans, hydraires, hydrozoaires, agua viva, hidra-morozoa,

hidrozoário, pólipo

Subclass Trachylina

Order Narcomedusae Haeckel, 1879

Suborder Narcomedusida Family Aeginidae

Genus Aegina Eschscholtz, 1829

Direct Children:

Aegina citrea Eschscholtz, 1829 - golf tee medusa Species

Species

Species

TIS taxonomy is based on the NODC Taxonomic Code **OCEANOGRAPHIC** 

last updated on 13 June 1996 (and not reviewed since...)

Other Source(s):

NODC Taxonomic Code, database (version 8.0) Source: Acquired:

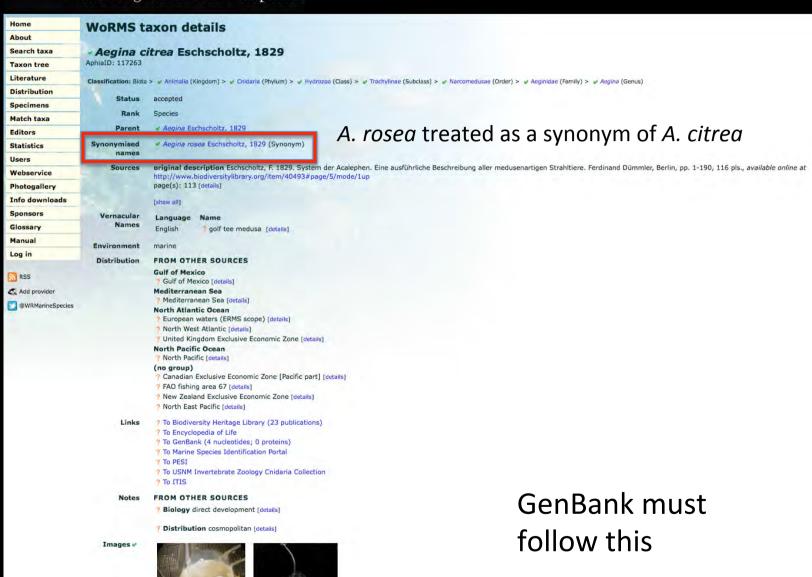
'Aegina rosea" of Collins et al 2008 following Bigelow, 1913





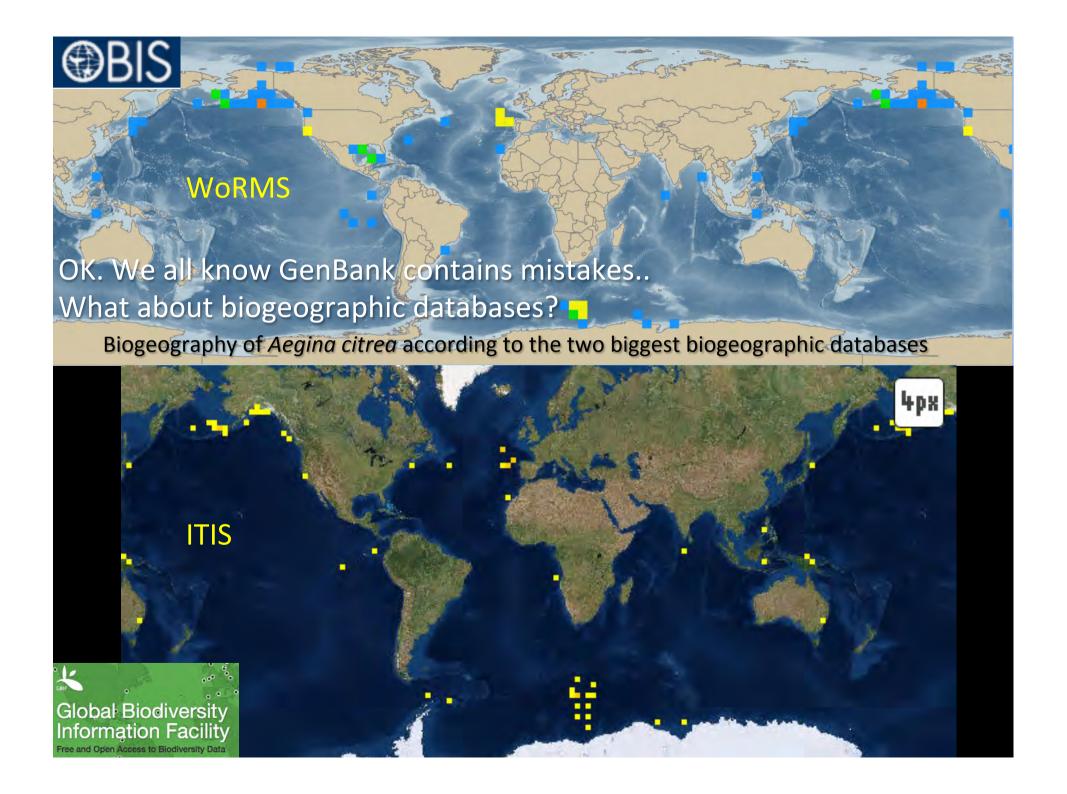


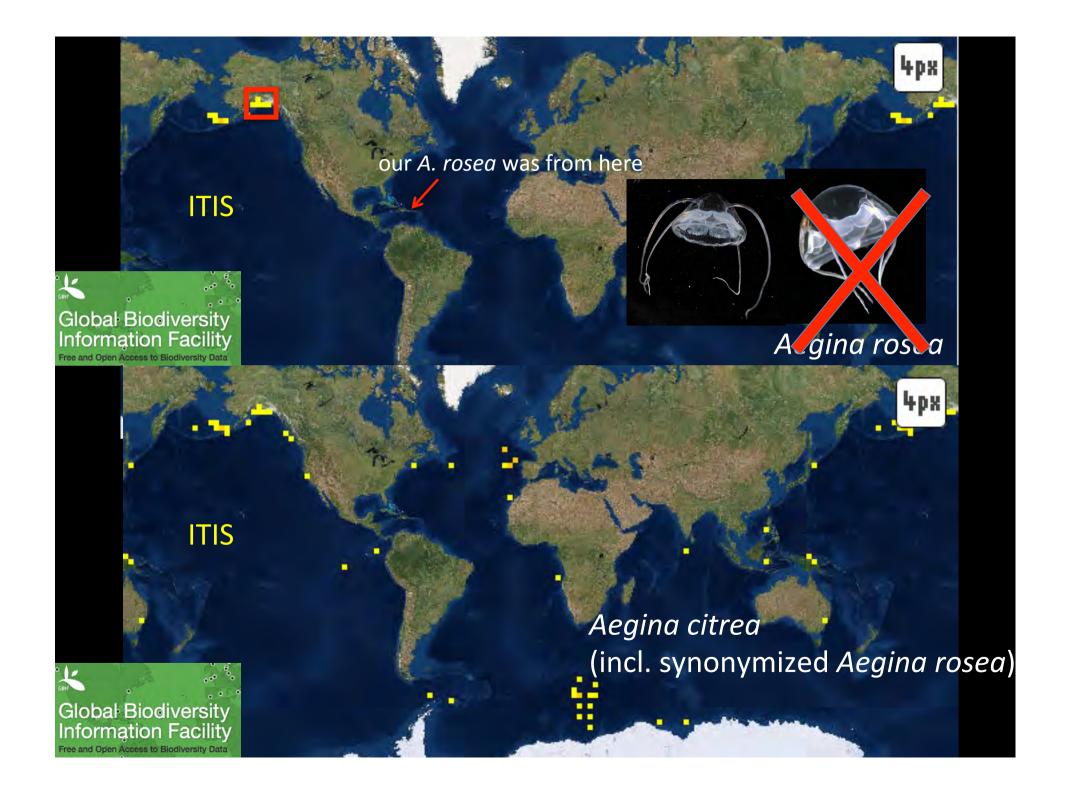


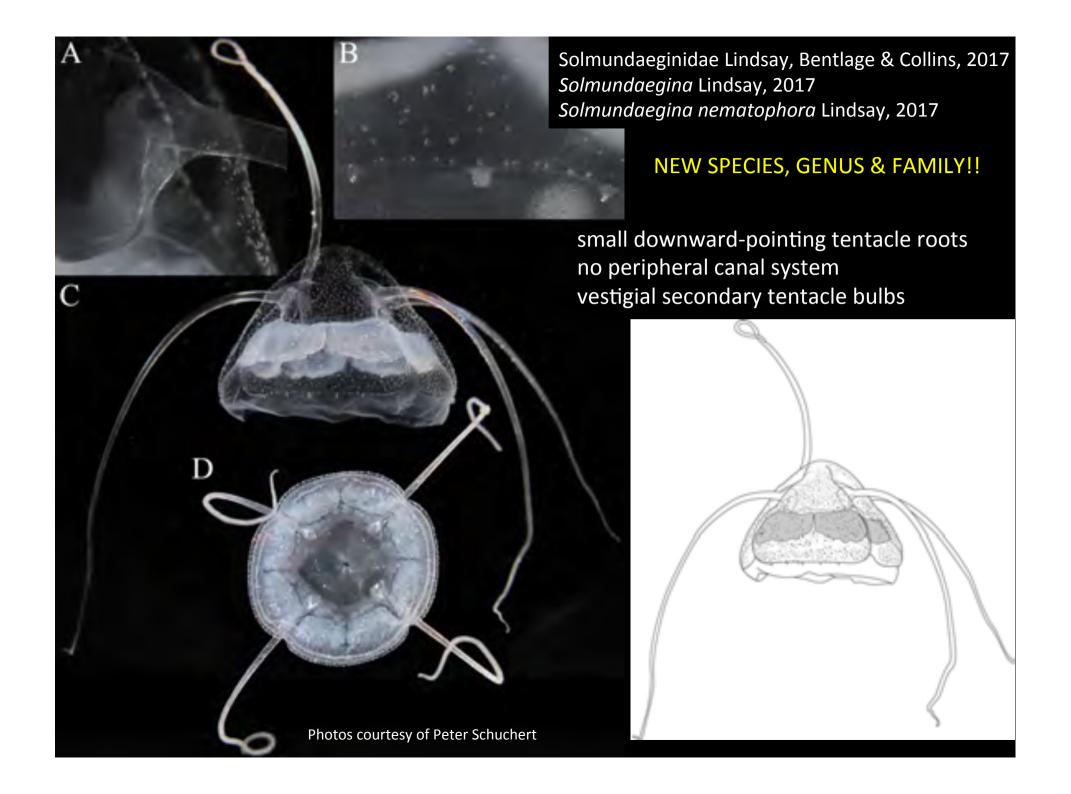


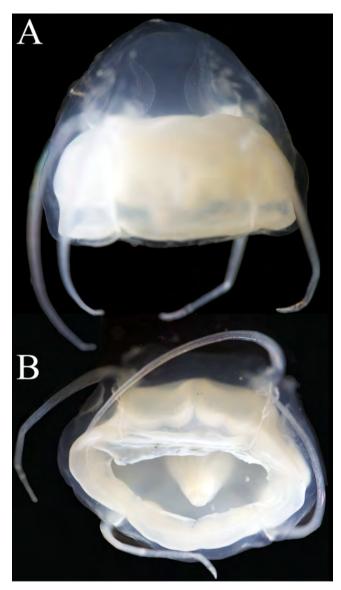
Aegina citrea





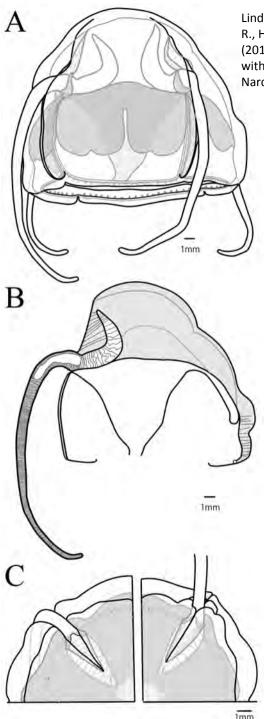




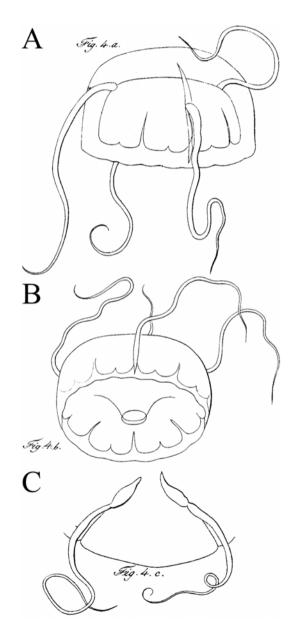


The REAL Aegina citrea! from near the Type Locality off eastern Japan

large upward-pointing tentacle roots peripheral canal system no vestigial secondary tentacle bulbs deep apical grooves

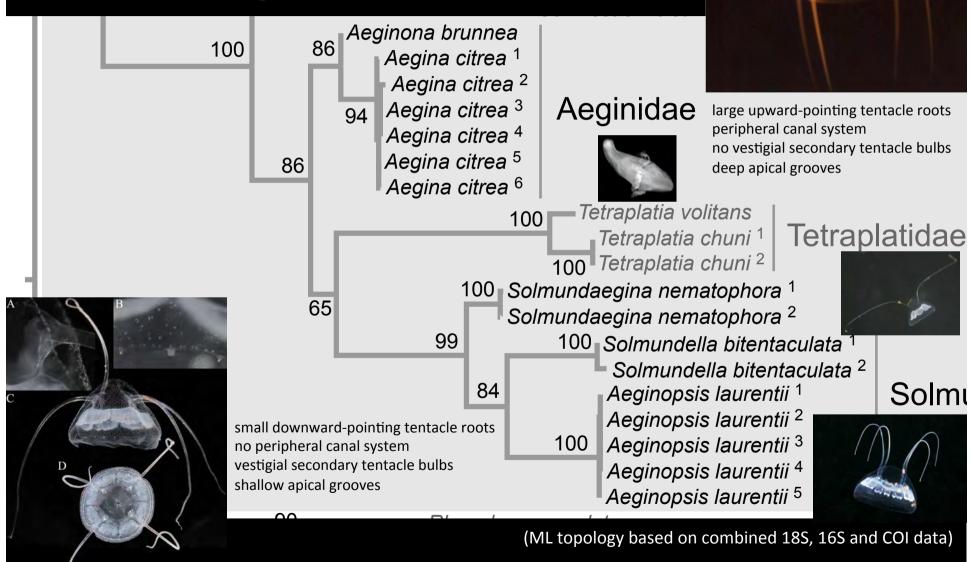


Lindsay, D.J., Grossmann, M.M., Bentlage, B., Collins, A.G., Minemizu, R., Hopcroft, R.R., Miyake, H., Hidaka-Umetsu, M. and Nishikawa, J. (2017) The perils of online biogeographic databases: A case study with the "monospecific" genus *Aegina* (Cnidaria, Hydrozoa, Narcomedusae). Marine Biology Research 13(5): 494-512.



The Collins et al 2008 sequence of "Aegina citrea" is actually the species *Solmundaegina nematophora* (in a different family)! Sorry....

What about the "Aegina rosea" of Collins et al 2008?



Pseudaegina Lindsay, 2017

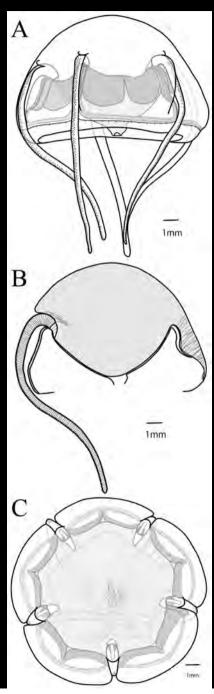


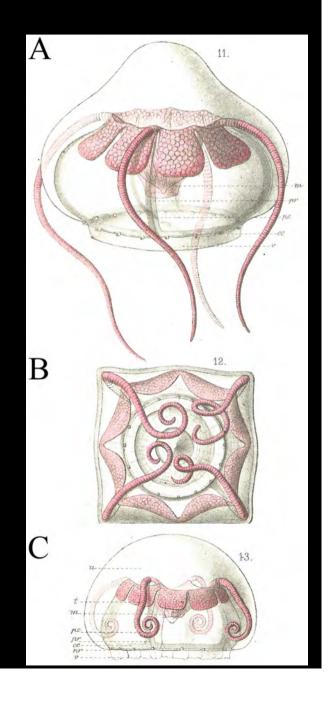
 $\mathbf{C}$ 

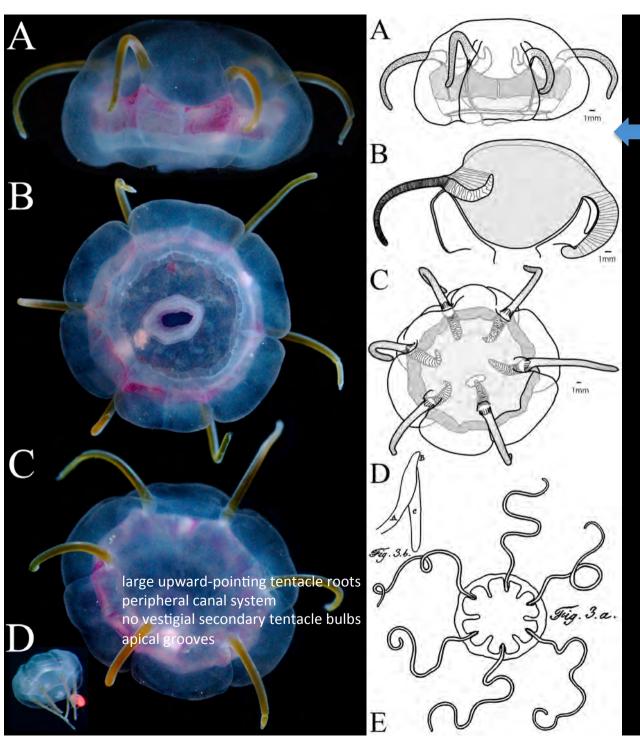
NEW GENUS & FAMILY!!

One of the Aegina citrea sequences in GenBank (the A. rosea of Collins et al 2008) is actually this species.

small downward-pointing tentacle roots no apical grooves peripheral canal system nematocysts only on upper surface of tentacles orally bulging mesoglea



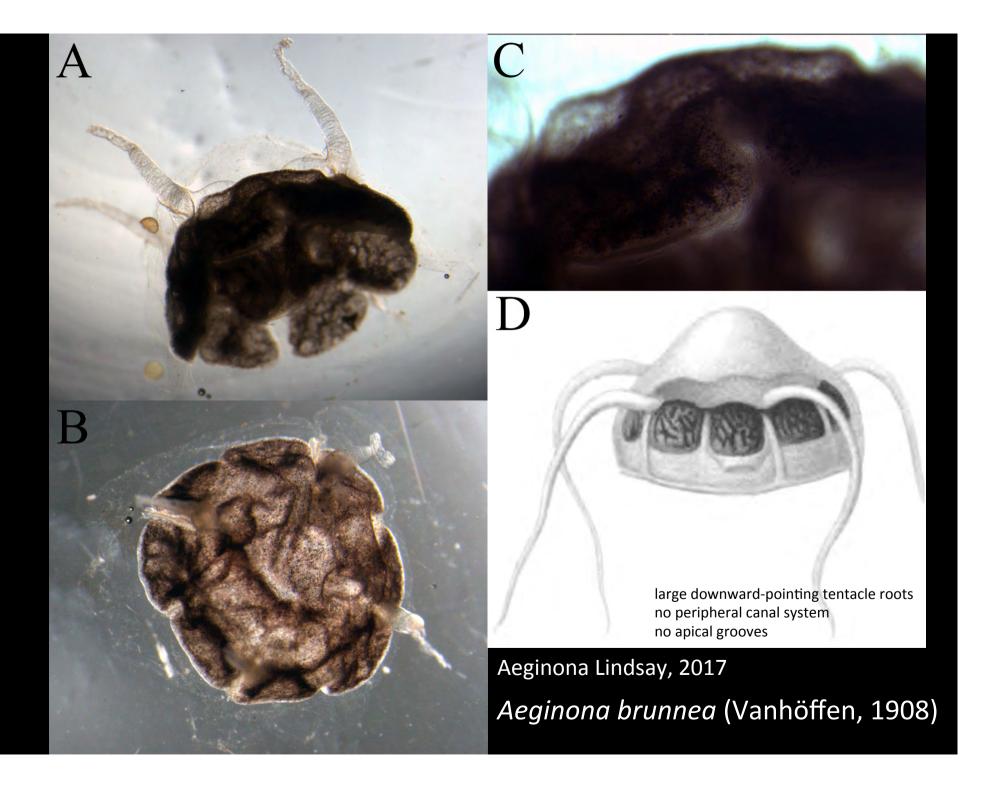


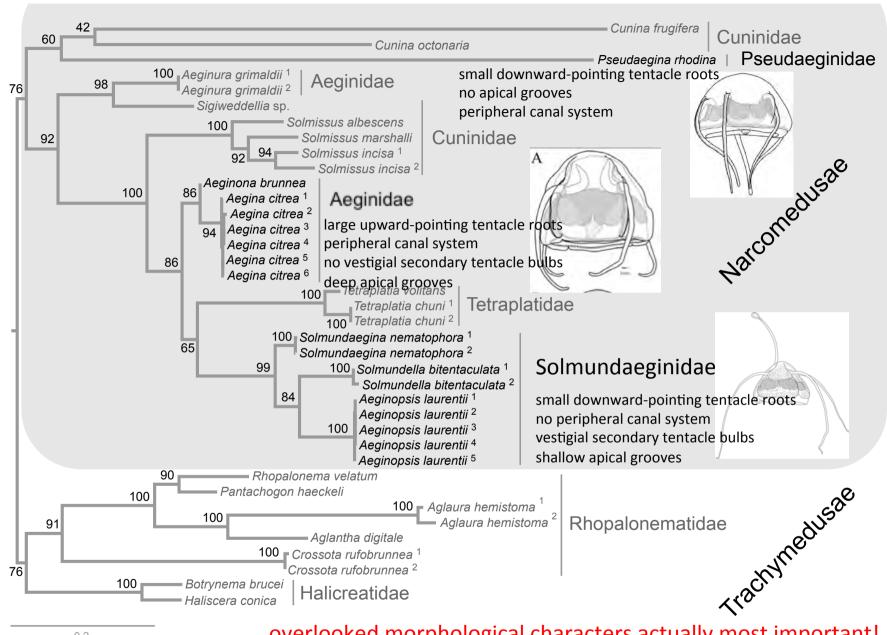


## The REAL Aegina rosea!



Therefore neither of the Collins et al 2008 sequences of (according to GenBank) "Aegina citrea" are actually that species. Furthermore, the species we labelled *A. rosea* actually belongs to a different family...





0.2 overlooked morphological characters actually most important! (ML topology based on combined 18S, 16S and COI data)

Lindsay, D.J., Grossmann, M.M., Bentlage, B., Collins, A.G., Minemizu, R., Hopcroft, R.R., Miyake, H., Hidaka-Umetsu, M. and Nishikawa, J. (2017) The perils of online biogeographic databases: A case study with the "monospecific" genus *Aegina* (Cnidaria, Hydrozoa, Narcomedusae). Marine Biology Research 13(5): 494-512.

• Aegina citrea was split into 6 separate species





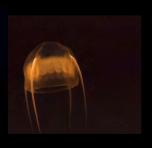






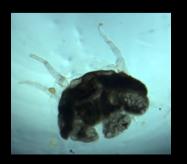
- Aegina citrea was split into 6 separate species
- Two new families, 3 new genera and 1 new species was described



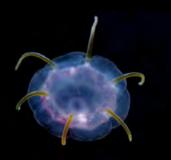


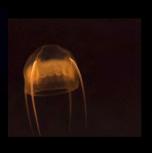






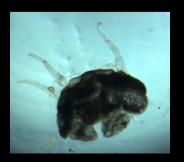
- Aegina citrea was split into 6 separate species
- Two new families, 3 new genera and 1 new species was described
- All Aegina citrea sequences in GenBank actually belonged to different species in different families to the real Aegina citrea





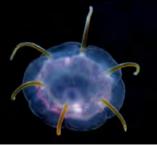






- Aegina citrea was split into 6 separate species
- Two new families, 3 new genera and 1 new species was described
- All Aegina citrea sequences in GenBank actually belonged to different species in different families to the real Aegina citrea
- >90% of records in biogeographic databases are wrong

The data compromised by not having good taxonomy and systematics was not only biogeographic in nature, but also led to erroneous data on predator-prey interactions (e.g. Mills & Miller 1984, Choy et al. 2017), parasite—host associations (e.g. Gasca et al. 2007), behaviour and life history strategies (e.g. Larson et al. 1989), vertical distributions (e.g. Arai & Mason 1982), environmental factors driving distributions (e.g. Luo et al. 2014) and DNA barcode sequences (e.g. Collins et al. 2008). This case clearly illustrates how systematics and taxonomy provide the foundation upon which all other biological science is built.











## Recommendations

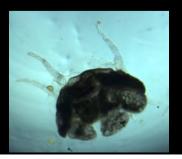
- Archive original data tables on biogeographic database servers with digital object identifiers (DOIs) to ensure data traceability because we were unable to verify most of the data in OBIS
- Always keep a voucher specimen of whatever you sequence.

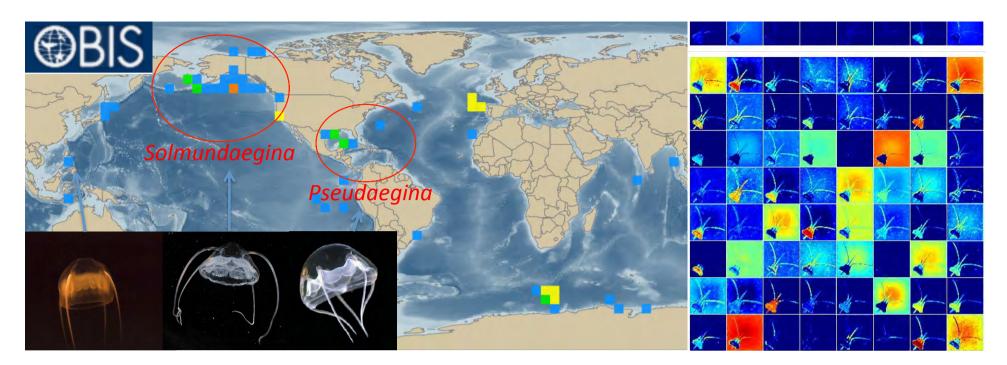








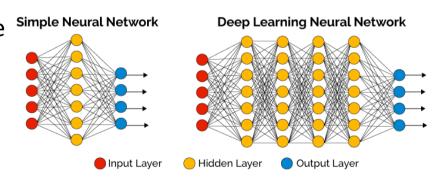




## What are their REAL distributions?

How to check and fix biogeographic database species IDs in light of Access and Benefit Sharing (ABS) policy/legal uncertainty?

Possible to use photos from SCUBA divers and ROV videos to assess?



Too many images for a human to work through...
Can a Deep-Learning Machine Vision AI approach be used?

## Deep Learning Al

NVIDIA Deep Learning GPU Training System (DIGITS) with CAFFE framework

```
2017-07-14 09:43:13 [INFO ] Loaded 47 jobs.
2017-07-14 09:43:13 [WARNING] Failed to load 3 jobs.
2017-07-14 09:43:13 [DEBUG] 20170628-093457-22a5 - IOError: [Errno 2] No such fi
le or directory: '/usr/local/DL-Box/digits-4.0/digits/jobs/20170628-093457-22a5/
|2017-07-14 09:43:13 [DEBUG] 20170704-105644-afbe - IOError: [Errno 2] No such fi
le or directory: '/usr/local/DL-Box/digits-4.0/digits/jobs/20170704-105644-afbe/
status.pickle'
2017-07-14 09:43:13 [DEBUG] 20170704-105938-97f4 - IOError: [Errno 2] No such ft
le or directory: '/usr/local/DL-Box/digits-4.0/digits/jobs/20170704-105938-97f4/
status.pickle
 * Running on http://0.0.0.0:34448/
127.0.0.1 - - [2017-07-14 09:43:19] "GET / HTTP/1.1" 200 29666 0.100632
127.0.0.1 - - [2017-07-14 09:43:19] "GET /static/css/bootstrap.min.css HTTP/1.1"
 304 190 0.007769
127.0.0.1 - - [2017-07-14 09:43:19] "GET /static/js/bootbox.min.js HTTP/1.1" 304
127.0.0.1 - - [2017-07-14 09:43:19] "GET /static/css/bootstrap-theme.min.css HTT
P/1.1" 304 189 0.003185
```

2,4,6 tentacles at a variety of angles as would occur in plankton net samples



Choice of network?

GoogleNet [22 layers]
gave better results
than
AlexNet [8 layers]
(but still not great..)

70-80% accuracy

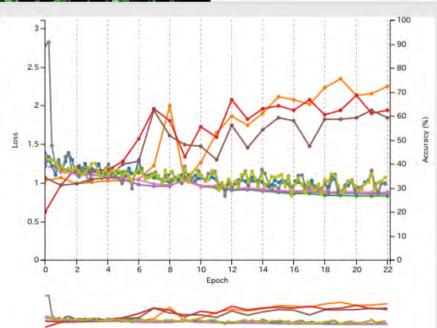


Image Mean:

#### **GPU Usage**

TITAN X (Pascal) (#0)

#### Memory

3.85 GB / 11.9 GB (32.4%)

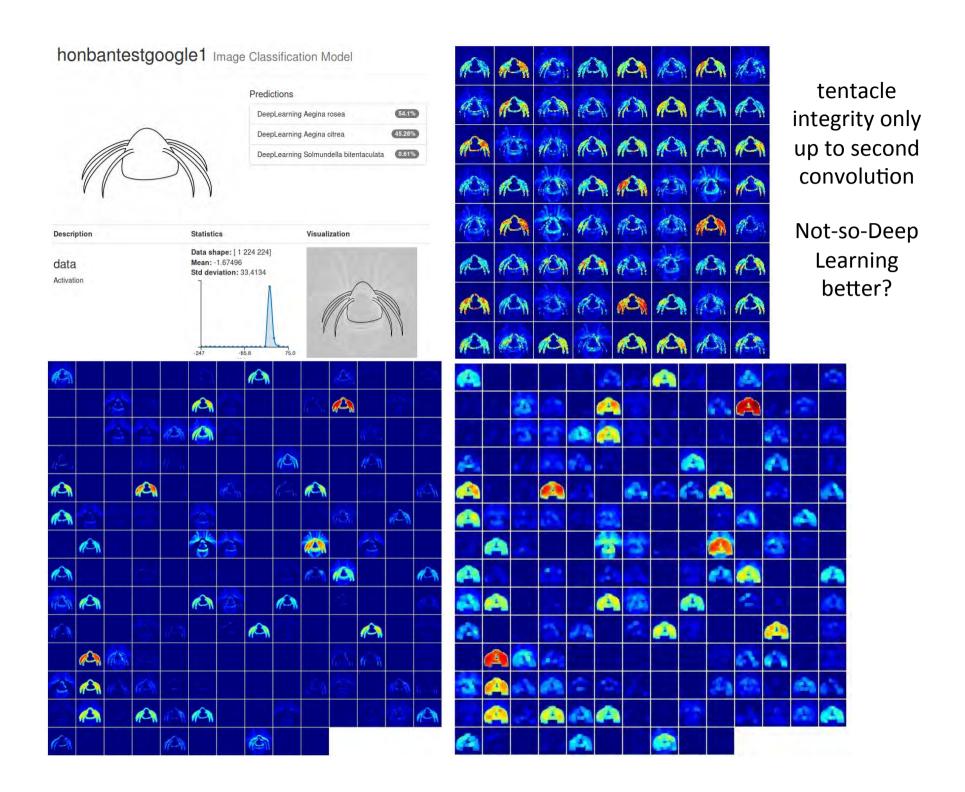
#### **GPU Utilization**

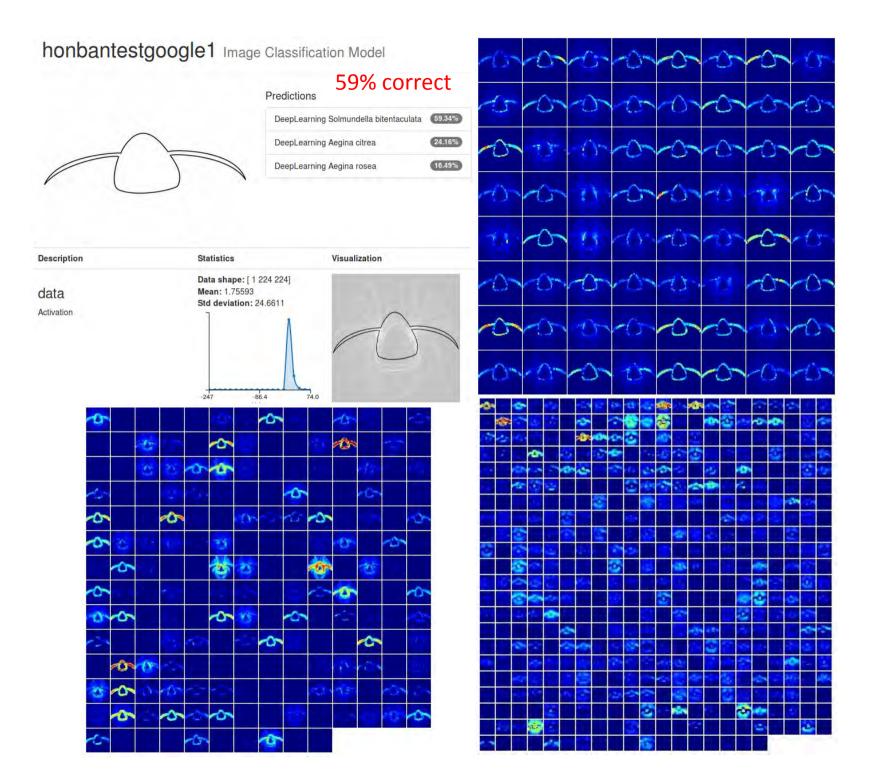
96%

#### Temperature

50 °C

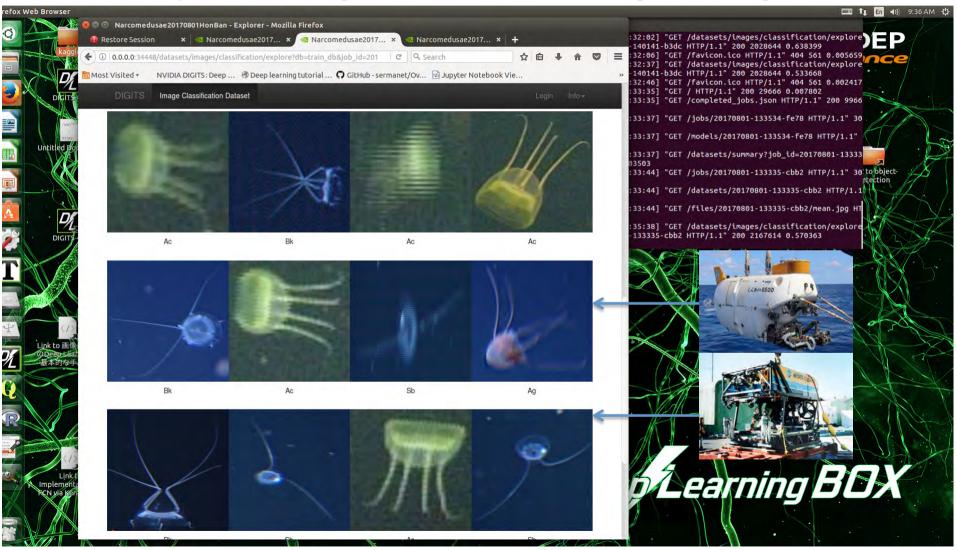






## Made an in situ image training set for 2-6 tentacle Narcomedusae using framegrabs from JAMSTEC ROV & HOV videos

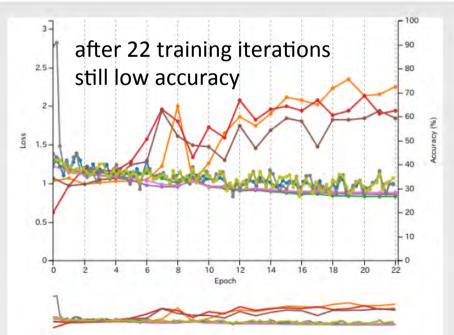
convert videos to still frame series, extract jellies manually with proprietary software, flip and rotate images to increase number of images in training set

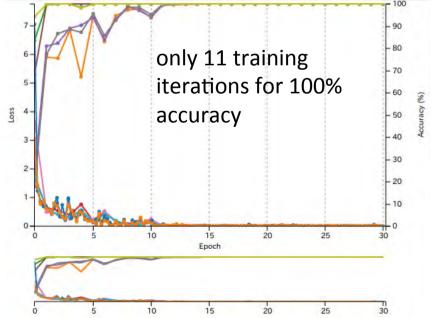


500-700 images per species



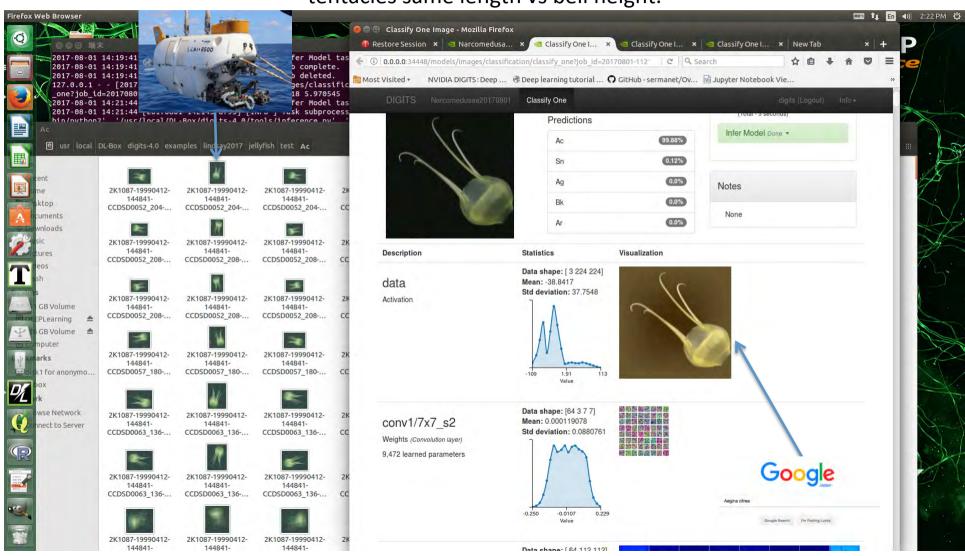






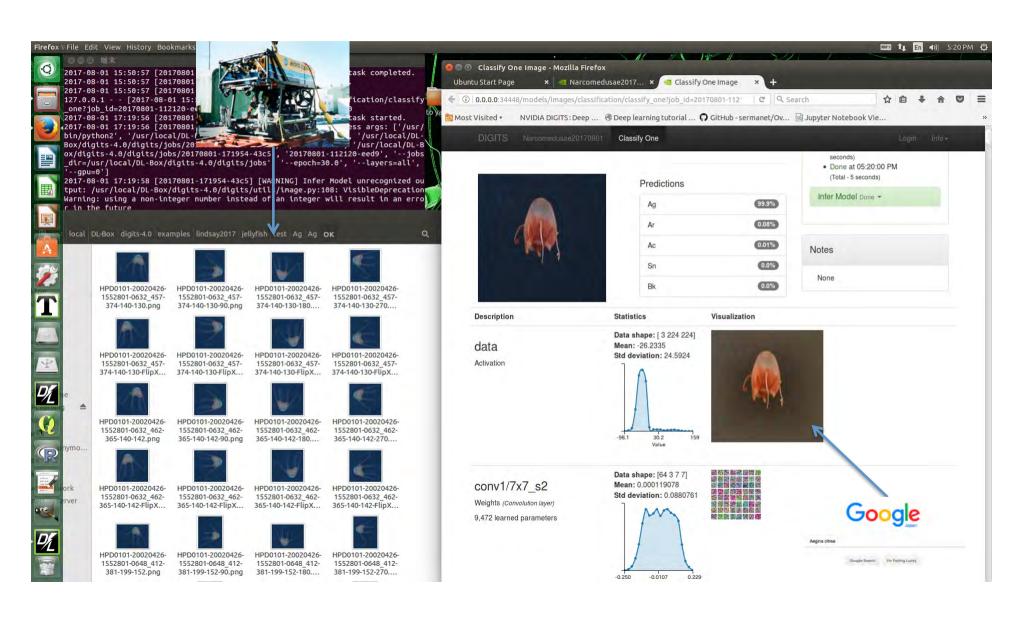
## Tried with "real" Aegina citrea framegrabs. Classified image from internet with 99.9% accuracy!!

When alive is yellow, always has tentacles forward and not touching each other, tentacles same length vs bell height.



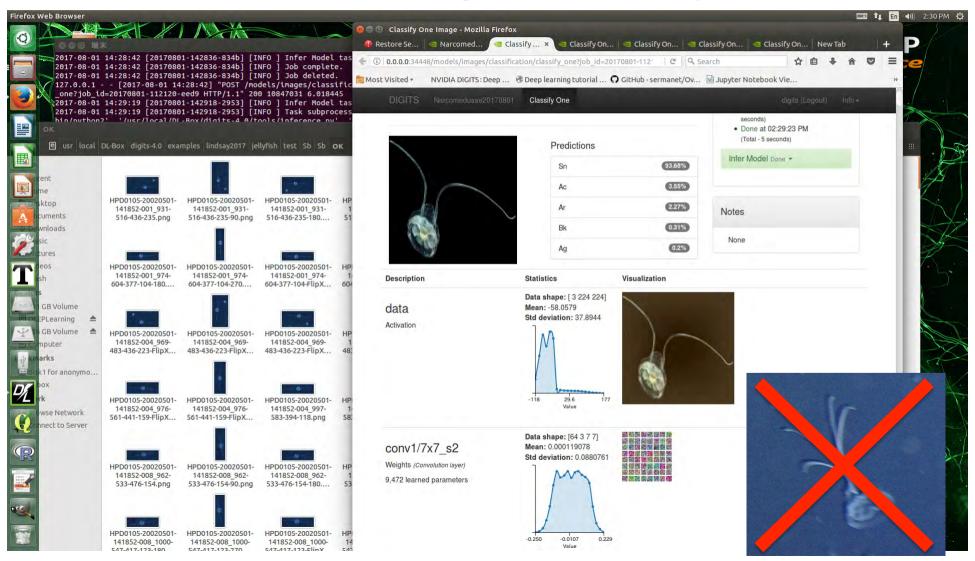
#### Aeginura grimaldii also 99.9% accuracy!!

When alive is brown-red and tentacles always sideways or down.



When the 2-tentacled *Solmundella bitentaculata* was <u>not</u> in the training set it was <u>misidentified</u> as *Solmundaegina nematophora* (same family) at 94% certainty level.

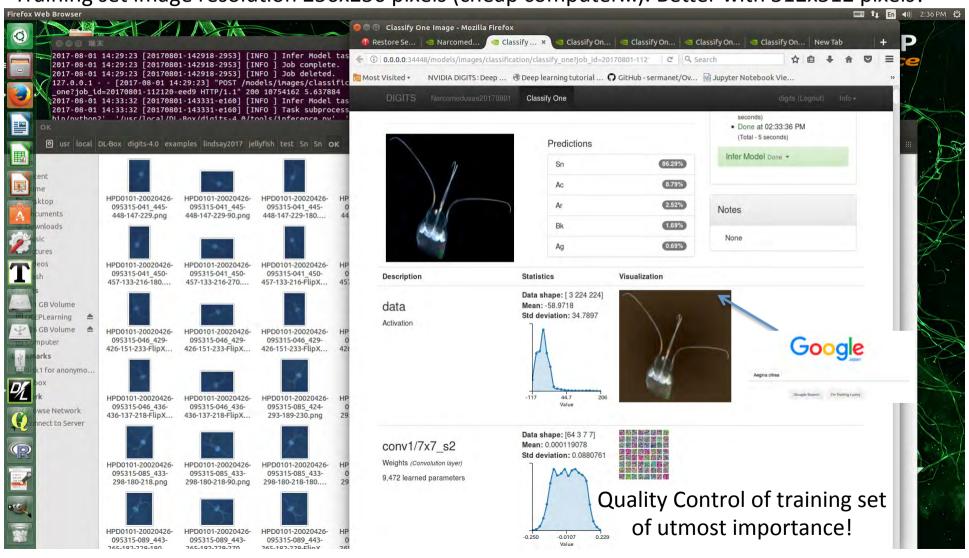
Need to ensure training set contains all species!!!



Solmundaegina nematophora was correctly identifed to 86% certainty (9% A. citrea).

Images do seem useful for automatic generation of species occurrence data from SCUBA photos or ROV images as a result of this taxonomic work

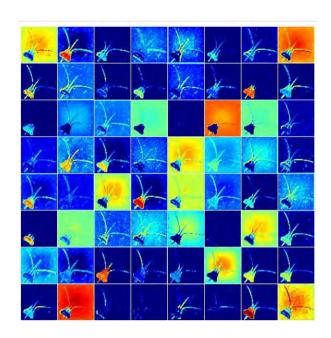
Training set image resolution 256x256 pixels (cheap computer...). Better with 512x512 pixels?



## Summary

If a "set" of in situ imagery and pristine specimens is collected for taxonomic work on similar-looking species, it appears that Deep Learning techniques will allow highly accurate classification of that taxonomic group from in situ video imagery.

(caveat: only tested with Narcomedusae so far)



accurate biogeographic data may be in our sights!

As with GenBank: Rubbish in  $\rightarrow$  Rubbish out

We all know eDNA needs more Groundtruthing but so do Deep Learning approaches











## Most up-to-date Taxonomy



Licandro, P., Fischer, A. and Lindsay, D.J. (2017) Cnidaria: Scyphozoa and Noncolonial Hydrozoa. in: Castellani, Edwards, M. (Eds.) Marine Plankton: A Practical Guide to Ecology, Methodology, and Taxonomy. Oxford University Press. 198–231. ISBN 9780199233267.

Licandro, P., Carré, C. and Lindsay, D.J. (2017) Colonial Hydrozoa (Siphonophorae). in: Castellani, Edwards, M. (Eds.) Marine Plankton: A Practical Guide to Ecology, Methodology, and Taxonomy. Oxford University Press. 232–250. ISBN 9780199233267.

Licandro, P. and Lindsay, D.J. (2017)
Ctenophora. in: Castellani, Edwards, M. (Eds.) Marine Plankton: A Practical Guide to Ecology, Methodology, and Taxonomy. Oxford University Press. 251–263. ISBN 9780199233267.



A Practical Guide to Ecology, Methodology, and Taxonomy

Edited by Claudia Castellani & Martin Edwards

