

Trying to resolve the taxonomic confusion of *Paracalanus parvus* species complex (Copepoda, Calanoida) in the Mediterranean and Black Seas through a combined analysis of morphology, molecular taxonomy and DNA metabarcoding

OR

Looking for tiny hair on the ladies' legs!



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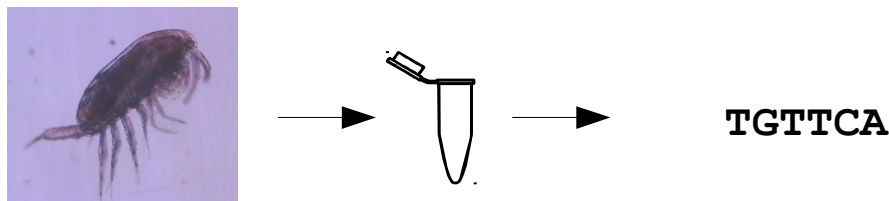
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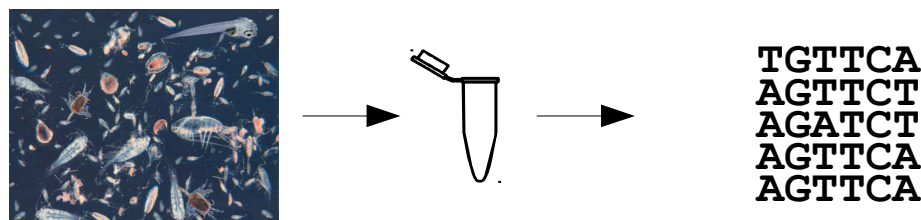
Overview

1) The *P. parvus* species complex and its taxonomic difficulties

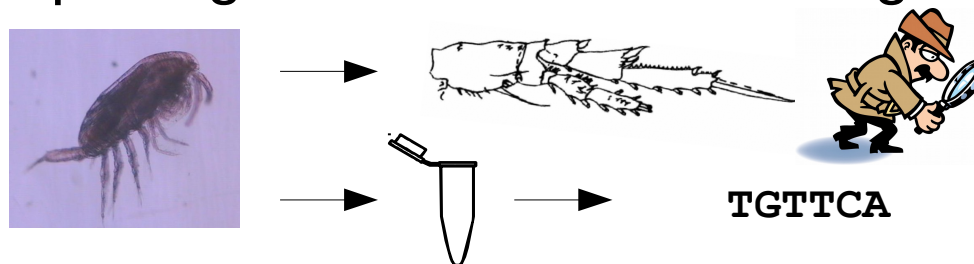
2) DNA barcoding (COI) to resolve taxonomy and geographic distribution



3) Additional data from DNA metabarcoding (16S rRNA)



4) Combined morphological and DNA barcoding analysis



5) Conclusions

The *Paracalanus parvus* species complex

- Species of *P. parvus* complex abundant in many marine ecosystems from temperate to tropical regions
- Consists of 7 morphological species

- *P. parvus*
- *P. indicus*
- *P. quasimodo*
- *P. nanus*
- *P. intermedius*
- *P. tropicus*
- *P. serrulus*

Taxonomic difficulties in distinguish among three, mainly between *indicus* and *quasimodo* (inconspicuous characters) (Bowman, 1971; Bradford, 1978; Kang, 1996; Ounissi and Khelifi-Touhami, 1999; Khelifi et al., 2007)

Morphological comparison of *P. parvus*, *P. indicus* and *P. quasimodo* (modified from Bradford, 1978)

Species	Outer distal edge of Ex3 serrated			B1 of P2-P4 with many posterior surface spinules	Authors
	P2	P3	P4		
<i>P. parvus</i> ♀	—	—	—	—	1,2,3
<i>P. parvus</i> ♂	?	?	?	—	2
<i>P. indicus</i> ♀	+	+	—	+	1,2,3,4
<i>P. indicus</i> ♂	+	+	+	+	2
<i>P. quasimodo</i> ♀	+	+	+	+	2
<i>P. quasimodo</i> ♂	+	+	+	+	2



B1: basipod 1, Ex3: exopod 3, Gns: genital segment, P2-4: leg2-4.

Authors 1: Bradford (1978), 2: Bowman (1971), 3: Kang (1996), 4: Sewell (1929)

Additional criteria (female) according to Bowman (1971)

Species	<i>P. parvus</i>	<i>P. indicus</i>	<i>P. quasimodo</i>
Body	Slender 0,85-0,90 mm	Slender-Shorter 0,9 mm	Stocky 1,0 mm
Dorsal hump	no	Slightly developed	Developed
Prosom/ Urosome	3,3-3,4/1	3,2/1	3/1
Gnsgm with posterior dorsal spinules	no	yes	yes
Spermatheca		Subelliptical Not narrowing distally	Obovate Narrowing distally
A1 reaches:	Midlength of caudal ramus	Anal segment	Posterior margin of caudal ramus



What was believed to be the distribution of *P. parvus* complex in the Mediterranean

- In the Mediterranean Sea, until recently, *P. parvus* was considered the dominant species.
- Bowman (1991) demonstrated that some specimens from several regions identified as *P. parvus* (Claus, 1863) were actually *P. indicus* Wolfenden, 1905 (Mediterranean, Atlantic and Indian Ocean) or *P. quasimodo* Bowman, 1971 (West Atlantic).
- In SW Mediterranean (Algeria, Tunisia) the identity of the copepod previously known as *P. parvus* was clarified and reassigned to *P. indicus* based on morphological analysis (Khelifi-Touhami et al., 2007).
 - *P. parvus* (Claus, 1863): Alboran Sea, SW Mediterranean (Balears, Algeria), Tyrrhenian Sea, Gulf of Naples, Adriatic Sea, Ionian Sea, Malta, Aegean Sea, Rhodes, Levantine Basin), Black Sea.
 - *P. indicus* Wolfenden, 1905: Alboran Sea, SW Mediterranean (Algeria), Gulf of Naples
 - *P. quasimodo* Bowman, 1971. Absent

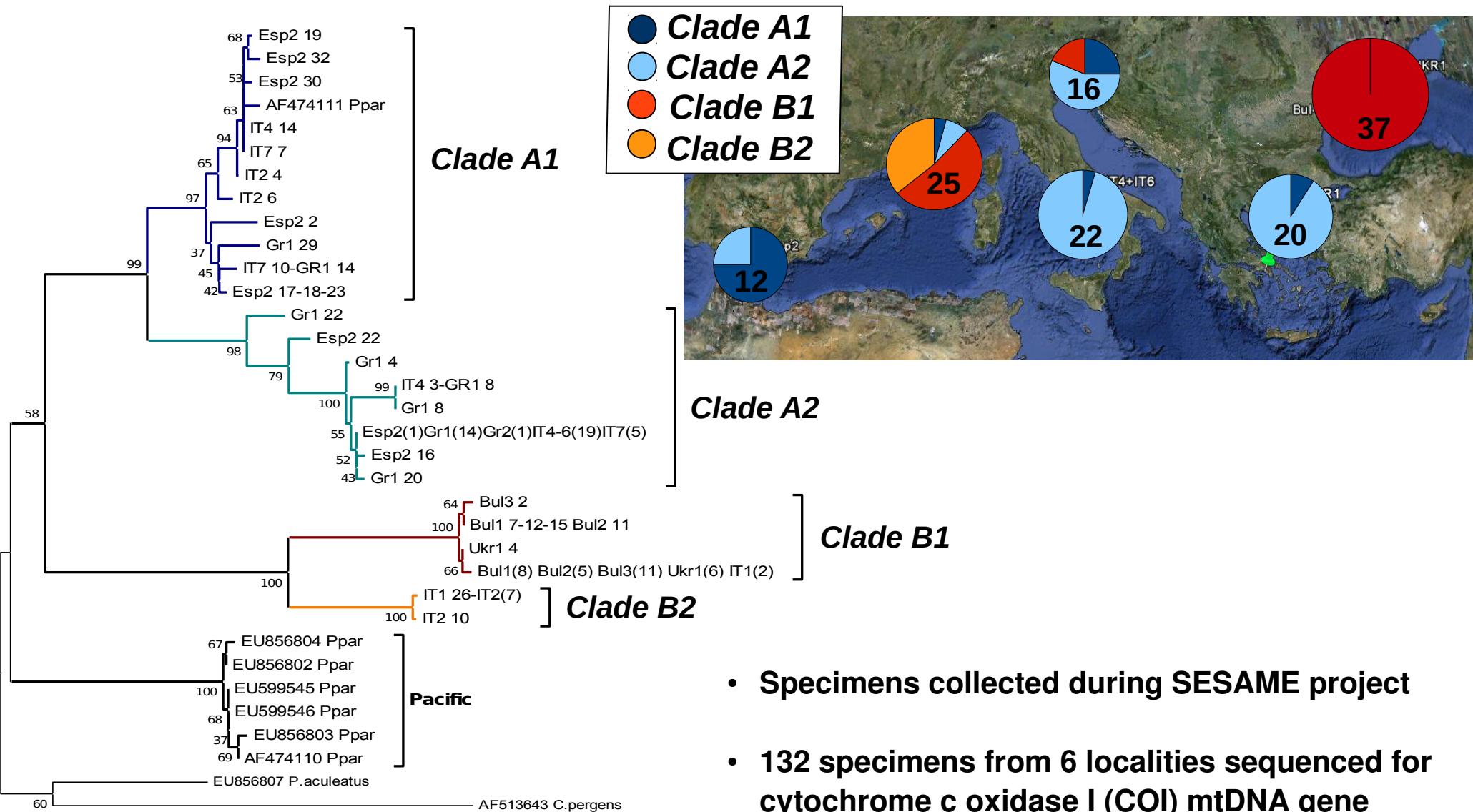
Doubts about the taxonomic status of *P. parvus* in the Mediterranean Sea

- The community of zooplanktonologists in North Mediterranean had indications from the morphology that what was considered as *P. parvus* may not be a single species.



- In 2008-9, *Paracalanus* samples were collected in the frame of the FP-6 EU project “SESAME” and genetically analyzed in HCMR, as a small side project.

First indications that *P. parvus* in the Mediterranean is not a single species



- Specimens collected during SESAME project
- 132 specimens from 6 localities sequenced for cytochrome c oxidase I (COI) mtDNA gene

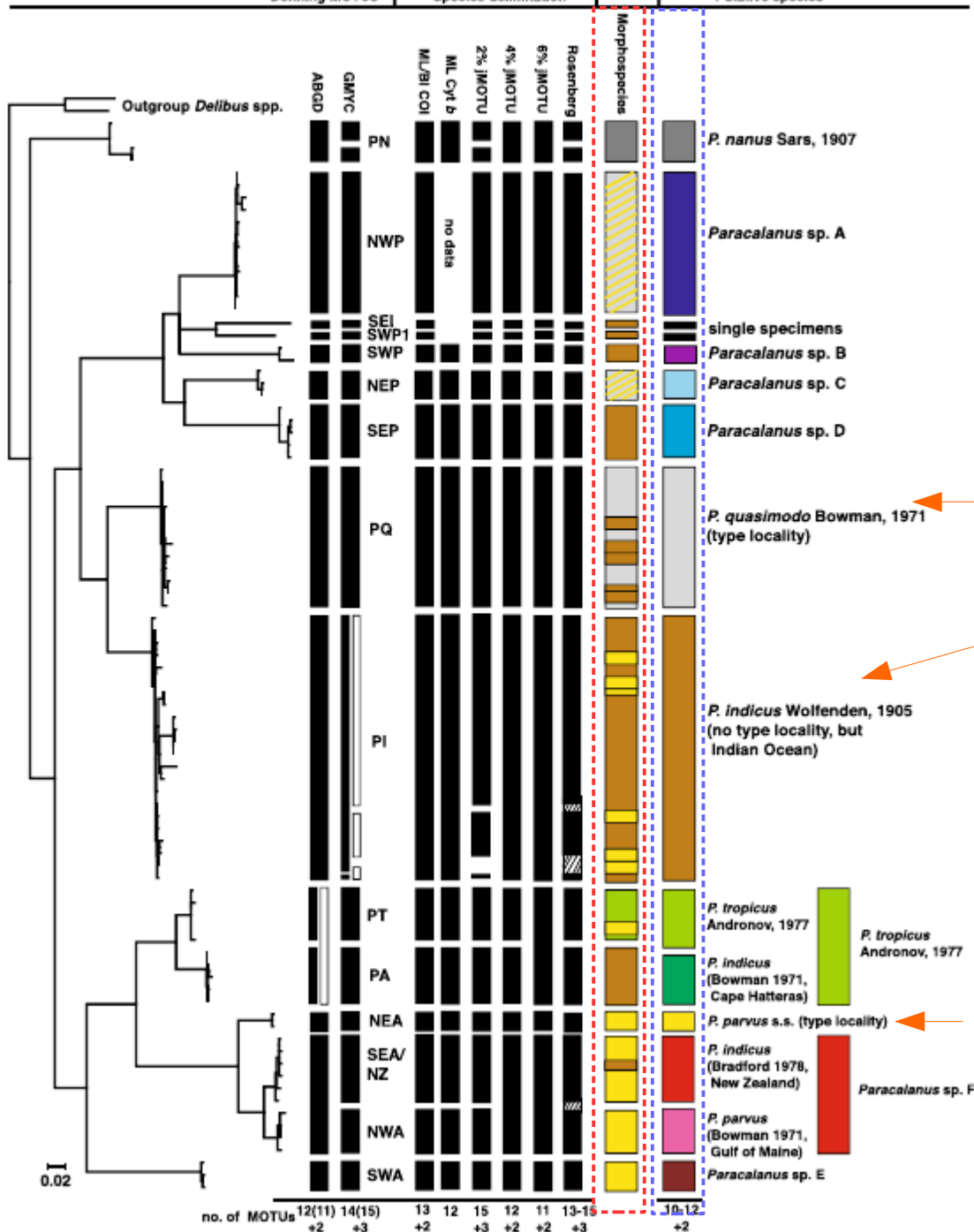


RESEARCH

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Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida)

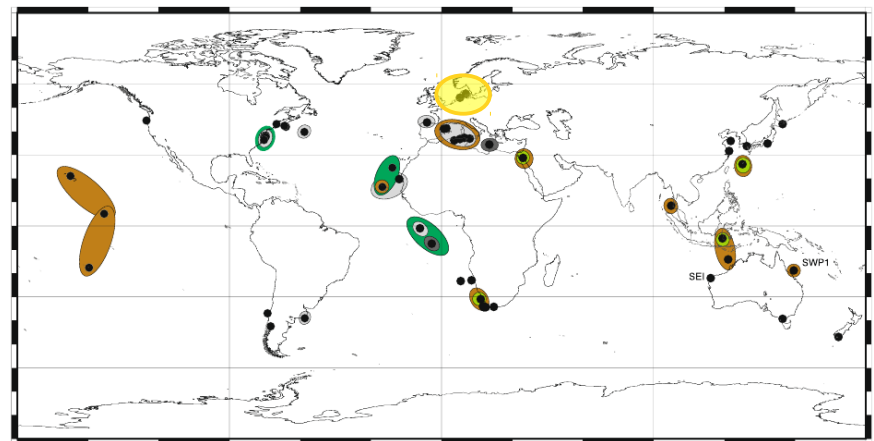
Astrid Cornils* and Christoph Held



Cornils & Held, 2014

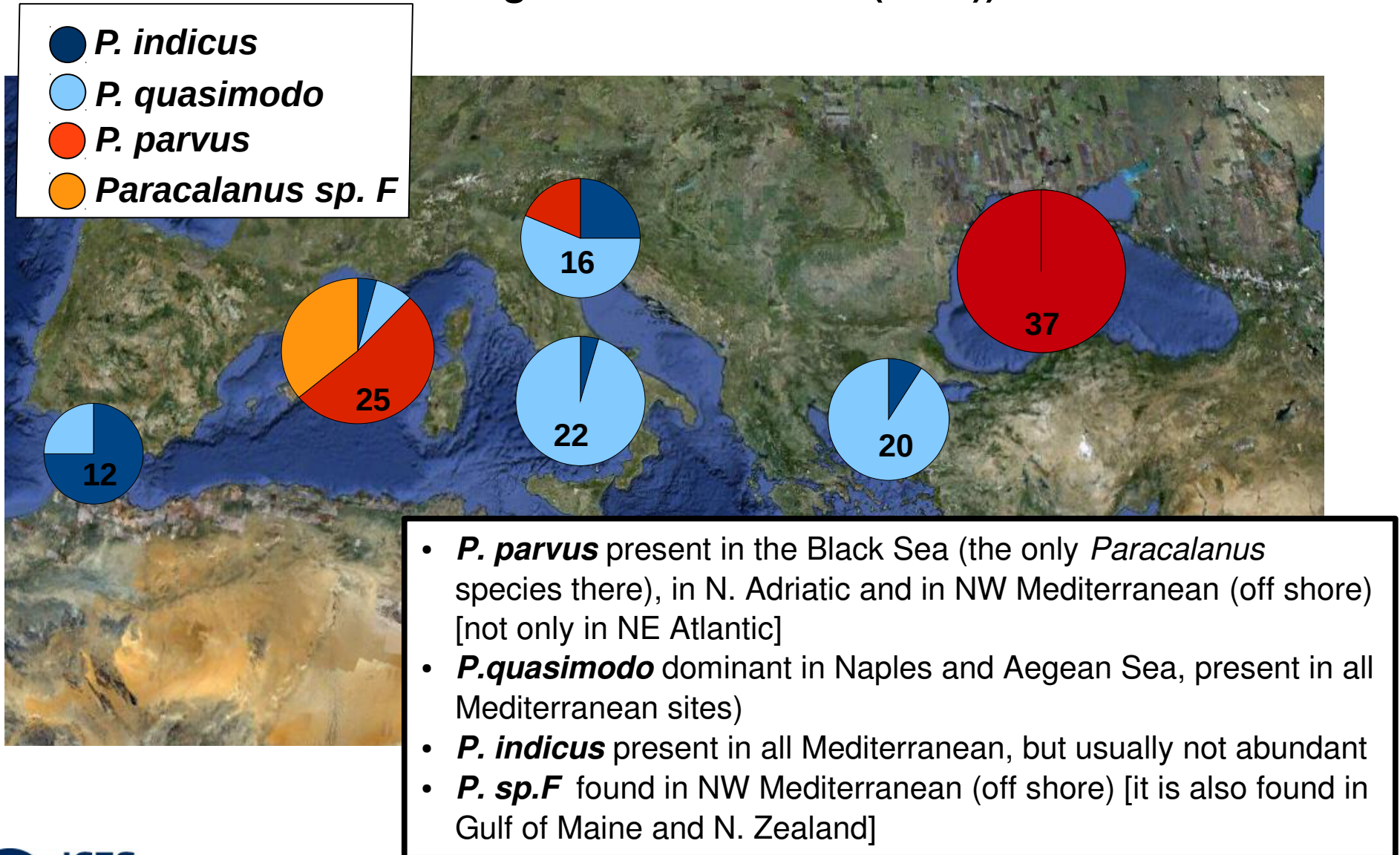
Main conclusions:

- *P. parvus* not a single species but 10-12 putative species with differing geographic distribution
- *P. parvus* s.s. only in NE Atlantic (type locality)
- *P. quasimodo* in Atlantic and W. Mediterranean (includes specimens identified by others as *indicus*)
- *P. indicus* has global distribution (including specimens from Pacific identified by others as *parvus*)



● *P. parvus* s.s. ● *P. quasimodo* ● *P. indicus*

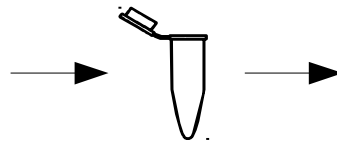
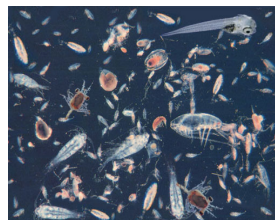
Distribution and relative abundance of species of *P. parvus* complex in Mediterranean and Black Sea (specimens from SESAME reanalyzed and assigned to species according to Cornils & Held (2014))





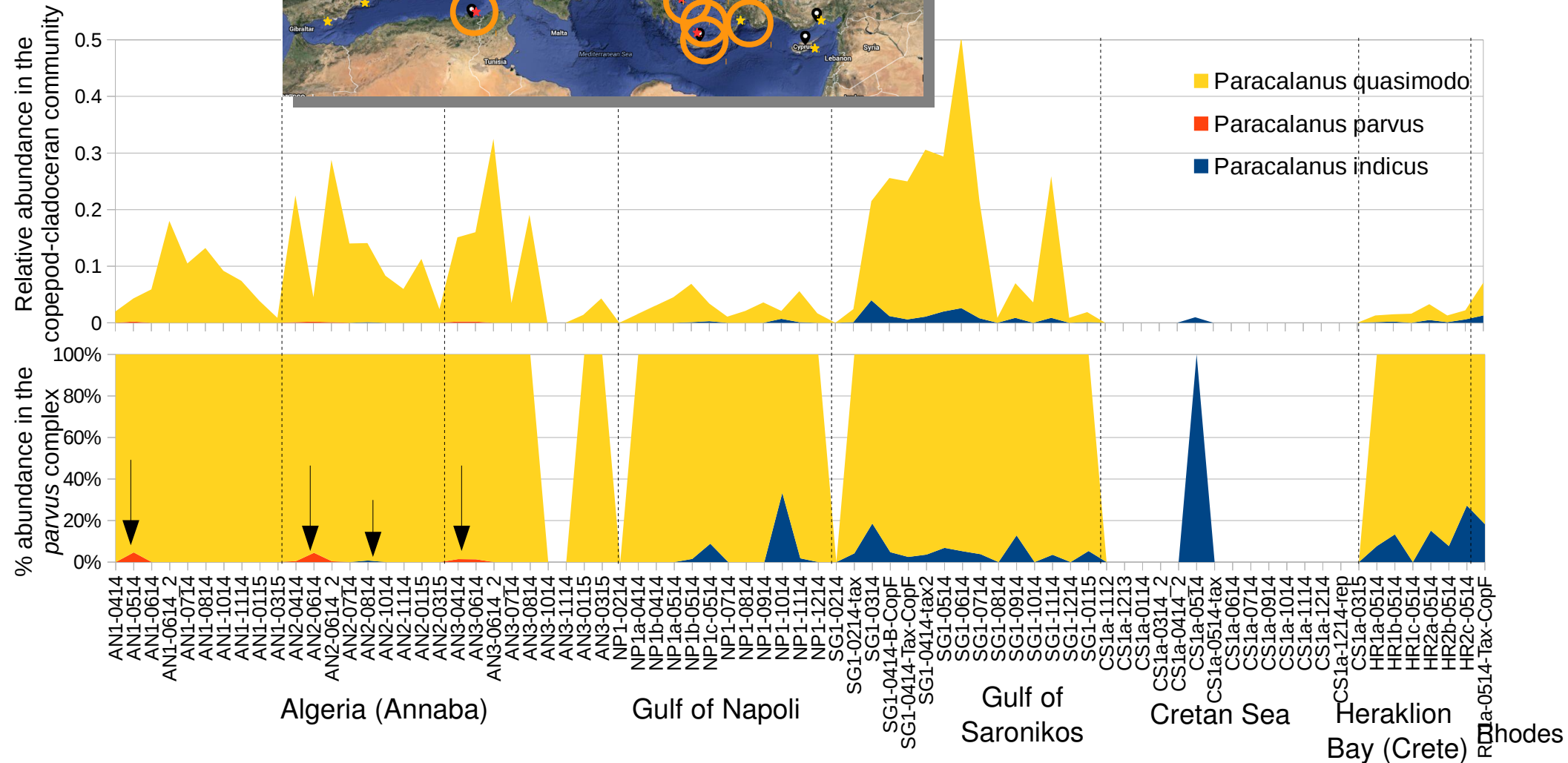
DNA metabarcoding analysis of bulk zooplankton samples

- “MetaCopepod” project: Develop a combined DNA metabarcoding and image analysis approach, to assess and monitor the biodiversity of marine planktonic copepods in a high-throughput, reliable and quantitative way.
- Methodology based on sequencing a portion of **16S rRNA** mitochondrial gene in Miseq (Illumina).
- A genetic reference database for the 16S rRNA was also produced for the main copepod species of the Mediterranean and the Black Sea for assigning MOTUs (all *Paracalanus* species are distinguished well).
- Several bulk zooplankton samples were processed, collected mostly from 4 monitoring areas (Algeria, Gulf of Naples, Gulf of Saronikos and Cretan Sea) at a monthly base.

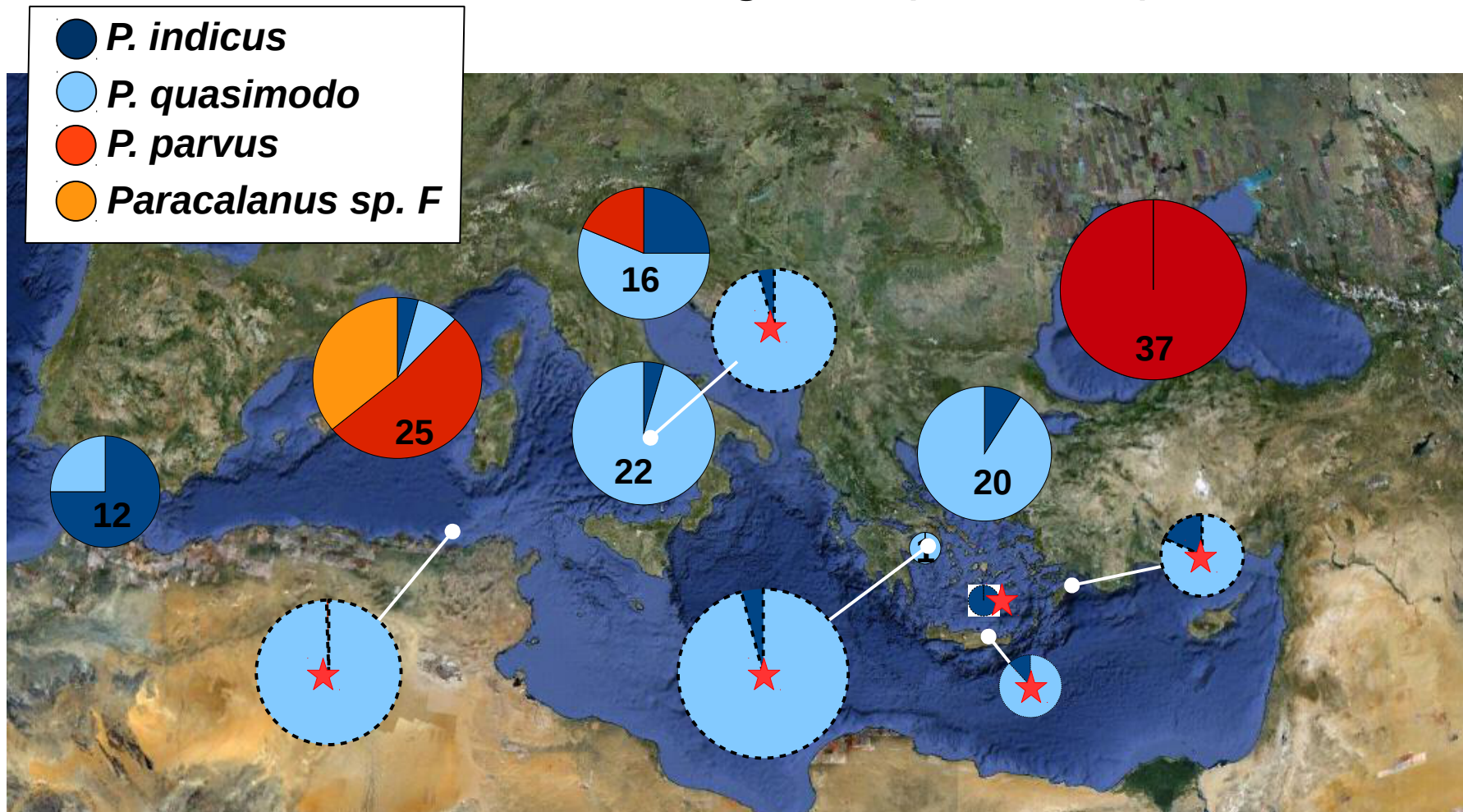


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TGTTCA  
AGTTCT  
AGATCT  
AGTTCA  
AGTTCA
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DNA metabarcoding results (16S rRNA) for the *P. parvus* complex in the Mediterranean Sea



Distribution and relative abundance of *P. parvus* complex based on COI sequences (Sanger) and DNA metabarcoding data (16S rRNA)



★ DNA metabarcoding data

➤ *P. quasimodo* dominant species in the coastal areas of the Mediterranean

Combined morphological and genetic analysis of *Paracalanus* samples from the Mediterranean Sea

- *Paracalanus* specimens collected from Algeria (6), Aegean Sea (Saronikos)(20), Napoli (6), N. Adriatic (26) and Black Sea (10)
- Identified under microscope; whole animal, legs P3 and P4 photographed.
- Same individuals then sequenced for COI and genetically identified.
- This work still in progress

Results of the combined morphological and genetic analysis (COI Sanger sequencing)

Algeria (Annaba):

All specimens (5) identified as indicus → quasimodo

Aegean Sea (Saronikos):

Specimens identified either as indicus (12) or parvus (6) → quasimodo

Napoli:

Specimens identified either as indicus or parvus → quasimodo

N. Adriatic:

Most of the specimens identified as indicus → quasimodo but two were indeed indicus


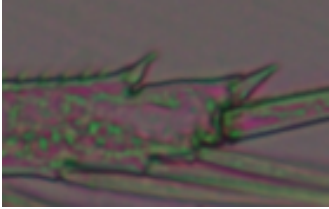
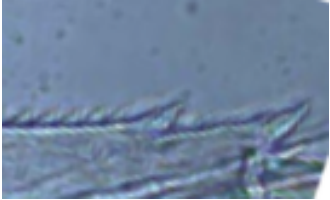
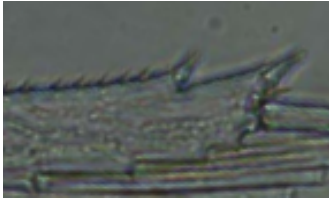

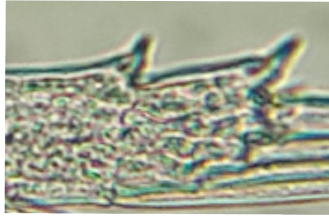
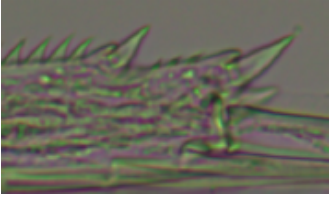
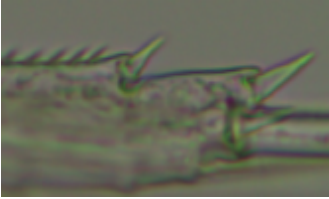
All specimens identified as parvus → parvus

Black Sea


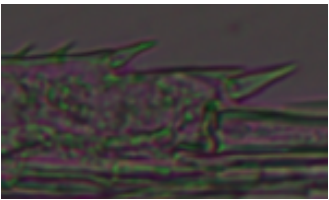



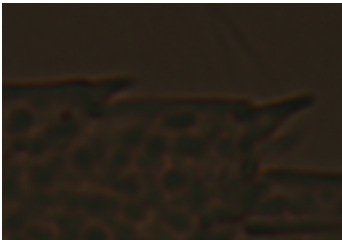
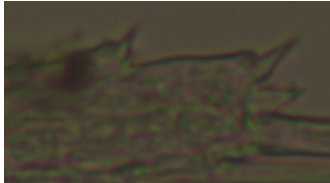
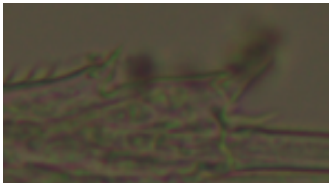
All specimens identified as parvus → parvus

- ***The genetic results in agreement with our genetic findings from SESAME and MetaCopepod.***

Indicative photos of specimens identified morphologically as *P. indicus*

	P3	P4	Morpho-species	Genetic species	Locality
P0178			<i>P. indicus</i>	<i>P. quasimodo</i>	Aegean Sea
P0185			<i>P. indicus</i>	<i>P. quasimodo</i>	Napoli
P0155			<i>P. indicus</i>	<i>P. quasimodo</i>	Algeria
P0223			<i>P. indicus</i>	<i>P. indicus</i>	N. Adriatic

Indicative photos of specimens identified morphologically as *P. parvus*

	P3	P4	Morpho-species	Genetic species	Locality
P0177			<i>P. parvus</i>	<i>P. quasimodo</i>	Aegean Sea
P0189			<i>P. parvus</i>	<i>P. quasimodo</i>	Napoli
P0211			<i>P. parvus</i>	<i>P. parvus</i>	N. Adriatic
SB3A-1			<i>P. parvus</i>	<i>P. parvus</i>	Black Sea

Conclusions

- Geographic distribution of the *P. parvus* species complex in the Mediterranean revised based on genetic data (barcoding and metabarcoding)
- Morphological characters used to discriminate the 3 species are not reliable.
- Further study is needed - use of nuclear markers to test for introgression and hybridization, detailed microscopy (SEM)
- More extensive sampling in the Mediterranean and the world oceans combined with DNA metabarcoding analysis could further elucidate the distribution of the *P. parvus* species complex.

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Thank you for your attention!