Strengthening marine amphipod DNA barcode libraries for environmental monitoring

Chinnamani Prasannakumar[1*], Ganesh Manikantan[2], J. Vijaylaxmi[3], Balakrishnan Gunalan[2,4], Seerangan Manokaran[5]

[1]Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Panaji, Goa-403004, India.

[2]Centre of Advance studies in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu- 608502, India.

[3]Department of Marine Sciences, Goa University, Taleigao Plateau, Goa-403206, India.

[4]Post Graduate and Research Department of Zoology, Thiru Kolanjiappar Government Arts College, Virudhachalam, Tamil Nadu- 606001

[5]Center for Environment & Water, King Fahd University of Petroleum and Minerals, Dhahran-31261, Saudi Arabia.

*Correspong author's email id: micropras@gmail.com

Abstract

Environmental DNA (eDNA) barcoding technology is finding innovative applications in monitoring terrestrial and marine biodiversity. DNA barcode reference libraries containing barcodes for wide range of species is vital for the success of monitoring any environments using eDNA barcodes. Since amphipods are used as bio-indicators for monitoring environmental quality and its health, we used DNA barcodes of amphipods to test the efficacy of present DNA libraries for species identification. We barcoded 22 amphipod species belonging to 17 genera of 13 families. 50% of barcodes produced in the present study was generated for the first time, as the sequences were absent in GenBank and Barcode of Life Databases (BOLD). Tree based identification method used in the present study precisely clustered the generated sequences with reference sequences. Besides exploring the distribution of recorded amphipod species, we show that with advent of next generation sequencing technologies, reference datasets such as ours will become essential for assessing health and monitoring various environments using amphipod barcodes.

Key words: Marine amphipods, Environmental monitoring, COI, DNA barcoding, Amphipod barcoding.

1. Introduction

Environmental DNA (eDNA) barcoding technology is finding innovative applications in monitoring terrestrial biodiversity (Heyde et al., 2020), marine biodiversity (Nguyen et al., 2020) including deep sea hydrothermal vents (Cowart et al., 2020) and zooplankton's gut content (Oh et al., 2020). eDNA barcoding technology has also proved efficient in environmental monitoring to detect invasive species (Kim et al., 2020) even from eDNA recovered from marine litters (Ibabe et a;., 2020). eDNA is the genetic material recovered from water, soil, or sediment (Taberlet et al. 2012; Thomsen and Willerslev 2015) and sequencing the barcode amplicon for revealing the taxonomy and biodiversity of the sampled environment is eDNA barcoding. Whereas DNA barcoding simply involves sequencing a gene fragment from precisely identified specimens to form a database and facilitate species identification (even by non-experts) simply by comparing the same gene sequences sequenced from unidentified specimens (Hebert et al., 2003, Mitchell, 2008).

eDNA technology had proven effective in spatial and temporal monitoring of wide range of environments as the techniques is relatively cheap, efficient and faster than traditional monitoring (Lecaudey et al. 2019; Preissler et al. 2019; Reinhardt et al. 2019; Sutter and Kinziger 2019; Sales et al. 2020). The innovation in eDNA barcoding technology is in its ability to monitor the environment without causing significant damage to the habitats or its species by non-invasive sampling strategy (Antognazza et al. 2019; Mora et al. 2019; Leempoel et al. 2020) and effectively detecting elusive, rare and cryptic species even in low density occurences
(Franklin et al. 2019; Shelton et al. 2019; Takahara et al. 2020). Single eDNA sampling could simultaneously monitor biodiversity over broad taxonomic spectrum in the given environment
(Sawaya et al. 2019; Thomsen and Sigsgaard 2019; Zhang et al. 2020).

Large DNA barcode reference library containing barcodes for broad range of species is vital for the success of monitoring any environments using eDNA barcoding. For example; while monitoring marine ecosystems, a previous study cannot assign >92% of eDNA amplicon sequences to any known phyla in the limited reference barcode library used and the sequences were described as unassigned species (Jeunen et al., 2019; Sawaya et al., 2019). A comprehensive, accurate reference library documenting individual species occuring locally with its photographic data along with its DNA barcodes are important for precise application of eDNA technology and success of such efforts were witnessed in eDNA barcoding of marine fishes (Stoeckle et al., 2020). Barcode of Life Database (BOLD)

(www.boldsystems.org) were created with the objective fulfilling above said requirements (Ratnasingham and Hebert, 2007).

Amphipods (Phylum: Arthropoda, Class: Malacostraca, Order: Amphipoda) are a significant invertebrate fauna associated with coastal ocean environments linking producers and consumers (such as fishes) in marine trophic webs (Sanchez-Jerez et al. 1999; Zakhama-Sraieb et al. 2006; Fernandez-Gonzalez and Sanchez-Jerez 2014). Amphipods were used as key organisms in assessing the environmental quality as they inhabit in close proximities with marine and estuarine sediments (Chapman et al., 1992, Chapman et al., 2013, Postma et al., 2002) and are used as a pollution bioindicator as they were sensitive to changes in environmental conditions (Bellan-Santini 1980; Virnstein 1987; Conradi et al. 1997; Guerra-Garcia and Garcia-Gomez 2001). Conventional taxonomy struggles for identifying amphipods as they were small with poor taxonomic descriptions and converged morphological characters (Knowlton, 1993, Radulovici et al., 2010), which make them an ideal group for application of DNA barcoding. DNA barcoding has been proven to work in marine amphipods of artic (Tempestini et al., 2018), Atlantic (Costa et al., 2009) and Pacific (Jażdżewska and Mamos, 2019) Oceans. However such efforts are rare in Indian Ocean area where amphipod diversity are richer (Mondal et al., 2010, Raja et al., 2013).

The objective of the present study is to identify amphipods occurring in sediments of Vellar estuary environments using DNA barcoding. The study also intent to test the efficacy of current DNA barcode reference libraries in identifying generated DNA barcodes, so that indirectly justifying reference library's ability in the future monitoring of environmental quality using eDNA barcodes.

2. Materials and methods

2.1. Sample collection and identification

Samples were collected from the mangroves sediment beds in Vellar estuary (Latitude: 11° 29'N. Longitude: 79° 46'E.) (Southeast coast of India) during Feb, 2012. A total of 6 samplings were taken at multiple sites around the mangrove species; *Rhizopora annamalayana* (Seetharaman and Kandasamy, 2011) using 50cm^2 quadrat. The salinity of the seawater was 30ppt (measured using hand-held Brix refractometer). The sediment samples were passed through 0.5mm sieve with copious ambient seawater and sieved at the field. The amphipods and other fauna along with residual sediments were preserved in 95% molecular grade ethanol (Merck, India) and transported to laboratory. Whenever found necessary, duplicate specimens were preserved in 5-7% formaldehyde containing Rose Bengal for

microscope analysis. Amphipods were sorted and identified on the basis of their morphological characters to the lowest level possible using a Nikon Eclipse E200 compound microscope. When required, the taxonomic keys of Vinogradov et al. (1996), Martin and Davis, (2001) Bousfield (1978), Balasubrahmaniyan and Srinivasan (1987), Lyla et al. (1999) and Lowry & Myers (2017) which is publicly available through the World Amphipoda Database (Horton et al., 2019) was referred for specimen identification. Description of samples with its respective photographic documents was made available under the project "DNA barcoding marine amphipods" (tag; DBMA) published publically in BOLD (www.boldsystem.org).

2.2. DNA isolation, PCR and sequencing

The DNA was extracted using the DNeasy Blood & Tissue Kits (Qiagen) following the manufacturer's protocols with modification in usage of 1/10th of actual reagent volume. Individual amphipods >12 mm in length were extracted using two or three pereopod and the whole amphipod specimens which were <12 mm length were used as such for DNA extraction. Mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified (658 base pair) using the primer pair LCO1490 and HCO2198 (Folmer et al., 1994). PCR was performed using a reaction mixture volume of 25µl; 12.5µl of Taq PCR Master Mix (Invitrogen, India), 11µl distilled water, 0.5µl forward primer (10 µM), 0.5µl reverse primer (10 µM), and 0.5µl of the DNA template (50–80 ng/µl). PCR conditions were; initial denaturation for 2 min at 95 ºC, followed by 5 cycles at 94 ºC for 30 s, 46 ºC for 45 s, 72 ºC for 45 s and 35 cycles at 94 ºC for 30 s, 51ºC for 45 s, 72 ºC for 45 s, and a final elongation step at 72 ºC for 5 min. All PCR amplicons were verified on a 1.5% agarose gel and commercially sequenced at Macrogen (Seoul, South Korea).

2.3. DNA sequence analysis

Amphipod specimen sequencing efforts were repeated until at least one individual in every species documented were sequenced. The sequences were read and manually double checked using ChromasLite ver.2.1. Gaps within the DNA sequences were checked by translating DNA sequences into putative amino acid sequences in BioEdit ver. 7.9 (Hall, 1999) and aligned in Clustal X ver. 2.0.6 (Thompson, 1997). Properly aligned sequences were made available through GenBank under the accession numbers MT184213-MT184234. All sequences with meta-data were also could be accessed under the project title “DNA Barcoding Marine Amphipods” and/or using a unique tag ‘DBMA’ in BOLD.

The Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert, 2007) and GenBank (Benson et al., 2018) are also used as a reference libraries to identify the generated barcode sequences in the present study. COI sequences were compared with other DNA barcodes available in BOLD was referred through 'identification engine' in BOLD and through Basic Local Alignment Searching Tool (BLAST) (Altschul et al., 1990) tool in GenBank using a standard protocol of similarity searching (Hu and Kurgan, 2018). Molecular Evolutionary Genetic analysis (MEGA) ver. 4.1 (Kumar et al., 2018) was used for constructing neighbour-joining (NJ) tree using Kimura 2 parameters (K2P). Pair-wise distance analysis was performed using K2P distance in MEGA. A NJ tree was redrawn using Interactive Tree Of Life (iTOL) (Letunic and Bork, 2019) for better representation of tree based identification.

3. Result and discussion

3.1. Species composition

Total of 2869 amphipod individuals with full morphometric characteristics were retrieved. Morphological identification assigned the whole collection into 22 species (fig. 1), 17 genera, and 13 families in the order Amphipoda. List of identified species were given in the Table 1. Folmer's primer (Folmer et al., 1994) used in the present study effectively amplified all 22 species, avoiding the need for additional prime pairs like those required in sequencing Atlantic (Costa et al., 2009) and deep sea Pacific amphipods (Jażdżewska and Mamos, 2019). All sequences amplified from after PCR were positively verified as Amphipoda COI gene fragments via BLAST searches against GenBank.

Occurrences of *Ampelisca scabripes* (Walker, 1904), *Grandidierella* sp. (Coutière, 1904), *Orchestia* sp. (Leach, 1814) and *Talorchestia* sp. (Dana, 1852) documented in the present study was also previously reported by in Vellar estuary (Mondal et al., 2010). *Ampelisca scabripes* (Walker, 1904) has been known to occur in Indian esturine system since 1975 (Rabindranath, 1975) till date (Srinivas, 2019). Though occurrences of *Ampithoe ramondi* (Audouin, 1826) in South Pacific islands were reported as early as 1986 (Myers, 1986) their occurrences in the present study is not surprising as *A. ramondi* were known for its active feeding habitats towards leaves and seeds of sea grasses (Castejón-Silvo et al., 2019). Ampithoe rubricata (Montagu, 1808) are most common amphipods previously known as inhabitants of kelp forest environments (Norderhaug et al., 2003) and as active feeders of red algae (Norderhaug, 2004). *Chelicorophium madrasensis* (Nayar, 1950) are the continuous feeder recently reported to dominate amphipod composition of Cochin estuary sediments

along the southwest coast of India (Rehitha et al., 2019). They were also reported as a common inhabitant of Thailand mangrove forests (Wongkamhaeng et al., 2015). *Elasmopus rapax* (Costa, 1853) were known to occur in Venezuela coast (Zanders and Rojas, 1992) and its invasiveness is realized in Australian waters (Hughes and Lowry, 2010). Possible invasiveness of this species in Vellar mangrove environment may deserve further investigation.

Fig. 1: Circular NJ tree drawn using Kimura-2 parametric distance model employing the COI sequences represented by each species of amphipods retrieved in the present study. Members of same family grouped together and vice versa.

Gammaropsis maculata (Johnston, 1828) are the good indicator of environment of dynamic hydrological forces (Conradi, 2001) and are known to occur in Tunisian coast of North Africa (Zakhama-Sraieb, 2017). *Gammarus locusta* (Linnaeus, 1758) documented in the present was known for its cosmopolitan estuarine distribution and are predicted to be more reluctant to ocean acidifications in near future (Hauton, 2009). *Grandidierella megnae*

(Giles, 1890) are previously known to occur along Basrah coast of Iraq (Naser et al., 2010) and in Songkhla Lake of Thailand (Rattanama et al., 2010). The endemic occurrences of *G. megnae* may requires further investigation. Though *Isaea elmhirsti* (Patience, 1909) documented in the present study was rarely studied species, *I. montagui* (H. Milne Edwards, 1830) was known for its epibiotic relationship with crabs (obtaining food from detritus and crab faeces) (Parapar, 1997). *Melita nitida* (Smith, 1873) was reported to be an invasive species for Western Scheldt estuary (in Netherlands) which was likely transported through shipping (Faasse and van Moorsel, 2003). They were known bio-indictors of toxic and petroleum pollutants in sediments by developing abnormal brood plate setae in their bodies (Borowsky et al., 1997).

Microdeutopus stationis (Della Valle, 1893) are known to occur in Tunisian coast of North Africa (Zakhama-Sraieb, 2017) and are recorded abundantly in seagrass beds of Isles of Scilly in southwest England coast (Bowden, 2001). Herbivory nature of *M. stationis* may be the reason for its preferential inhabitation of Vellar mangrove environment. Occurence of *Platorchestia platensis* (Krøyer, 1845) previously known along the Swedish coast and also in the Baltic Sea (Persson, 2001). The ability of *P. platensis* to reproduce around the year was reasoned as one of its attribute for its invasiveness in warm temperate waters of South African estuary (Hodgson et al., 2014). *Othomaera othonis* (H. Milne Edwards, 1830) are known to occur along Portuguese continental shelf in northern Atlantic Ocean (Sampaio et al., 2016) and in continental shelf of Algeria, Mediterranean Sea (Bakalem et al., 2020). However its occurrences in shallow mangrove sediments in the present study was interesting and demands further investigation. *Protohyale honoluluensis* (Schellenberg, 1938) recoded in the present study was also known in occur in marine caves of Hong Kong Island (Horton, 2008). *Talorchestia martensii* (Weber, 1892) are commonly known as equatorial sandhoppers occur in beach sand of African (Ugolini, 2016) and Kenyan coast (Ugolini and Ciofini, 2015). *T. martensii* widely studied for its astronomical orientations (Ugolini and Ciofini, 2015; Ugolini, 2016). *Victoriopisa chilkensis* (Chilton, 1921) was known to occur in Malaysian coast (South China Sea) and were used as feed in Thailand shrimp culture (Yokoyma et al., 2002).

In the present study, four species viz., *Ampelisca* sp., *Dexamine* sp., *Isaea* sp., *Leptocheirus* sp., and *Pectenogammarus* sp. was not resolved to species level with neither conventional or molecular techniques. These barcodes in public databases might be resolved to species level in near future when the respective barcode from precisely identified species was obtained.

Table 1: Identification of COI sequences using GenBank and BOLD systems.

Species identified in the present study	**Closest species match in GenBank**	**BLAST similarity %**	**Accession numbers of closest match**	**BOLD best match**	**Top % of similarity in BOLD**
Ampelisca scabripes	*Ampelisca macrocephala*	95.92	MG313065	No match	

***Ampelisca* sp.**	*Ampelisca* sp.	98.21	KX223977	*Ampelisca* sp. AB2	100
Ampithoe ramondi	*Ampithoe ramondi*	99.26	KP316300	*Ampithoe ramondi*	100
Ampithoe rubricata	*Ampithoe rubricata*	98.53	HQ987379	*Ampithoe rubricata*	100
Chelicorophium madrasensis	*Chelicorophium robustum*	93.11	KM009063	No match	
***Dexamine* sp.**	*Dexamine thea*	97.89	KT209114	*Dexamine* sp. AB19	100
Elasmopus rapax	*Elasmopus rapax*	98.69	KX224028	*Elasmopus rapax*	100
Gammaropsis maculata	*Gammaropsis maculata*	100	MG935019	*Gammaropsis maculata*	100
Gammarus locusta	*Gammarus locusta*	99.35	MG935024	*Gammarus locusta*	100
Grandidierella megnae	*Grandidierella chaohuensis*	92.82	KT180187	No match	
Isaea elmhirsti	*Dulichiidae* sp.	90.51	MN346579	No match	
Isaea montagui	*Dulichiidae* sp.	88.87	MN346579	No match	
***Isaea* sp.**	*Dulichiidae* sp.	93.29	MN346579	No match	
***Leptocheirus* sp.**	*Leptocheirus pinguis*	96.07	MG318679	*Leptocheirus* sp. AB5	100
Melita nitida	*Melita nitida*	97.88	KF273656	*Melita nitida*	97.35
Microdeutopus stationis	*Microdeutopus* sp.	98.21	KX224078	*Microdeutopus chelifer*	97.54
Platorchestia platensis	*Platorchestia* sp.	93.63	MH279725	*Platorchestia platensis*	100
Othomaera othonis	*Othomaera othonis*	97.72	MG935257	No match	
***Pectenogammarus* sp.**	*Pectenogammarus planicrurus*	92.21	MK159963	No match	
Protohyale honoluluensis	*Protohyale cf. jarrettae*	95.43	MG319374	No match	
Talorchestia martensii	*Talorchestia martensii*	98.85	KC578515	*Talorchestia martensii*	100
Victoriopisa chilkensis	*Victoriopisa chilkensis*	100	MK526894	No match	

3.2.Barcode identification using reference library

Among the various COI sequences generated, the sequences of the families viz., Ampeliscidae (*Ampelisca* sp. (Krøyer, 1842), *A. scabripes* (Walker, 1904)), Corophiidae (*Chelicorophium madrasensis* (Nayar, 1950), *Leptocheirus* sp. (Zaddach, 1844)), Aoridae (*Grandidierella megnae* (Coutière, 1904)), Talitridae (*Platorchestia platensis* (Krøyer, 1845), *Talorchestia martensii* (Weber, 1892)), Hyalidae (*Protohyale honoluluensis* (Schellenberg, 1938)) and Isaeidae (*Isaea* sp. (H. Milne Edwards, 1830), *I. elmhirsti* (Patience, 1909) and *I. montagui* (H. Milne Edwards, 1830)) were barcoded for the first time. That is the barcodes of 50% of species retrieved in the present study was absent in reference barcode libraries. The COI sequences of the family Isaeidae generated in the present study was found to be sequenced for first time ever as no members belonging to this family was previously found in GenBank library. The COI barcodes were also cross referred in BOLD library, where all the sequences barcoded for first time was declared as “no match” by BOLD system (Table 1).

3.3.Tree based identification

Based on the statistical significance (percentage of identity, query coverage, e-value), reference sequences were retrieved from GenBank for tree based identifications. Sequences produced for the first time did not have significant match in the database and was used as such without any reference sequence in the NJ tree construction (Fig. 2). All COI sequences produced in the present study (n=22), clustered in same branch with the reference sequences (n=24) (Fig. 2). Most of the branches in the NJ tree was supported by significant (>75) bootstrap values. The references sequences used precisely clustered with the sequences produced in the present study indicating the success of tree based identification.

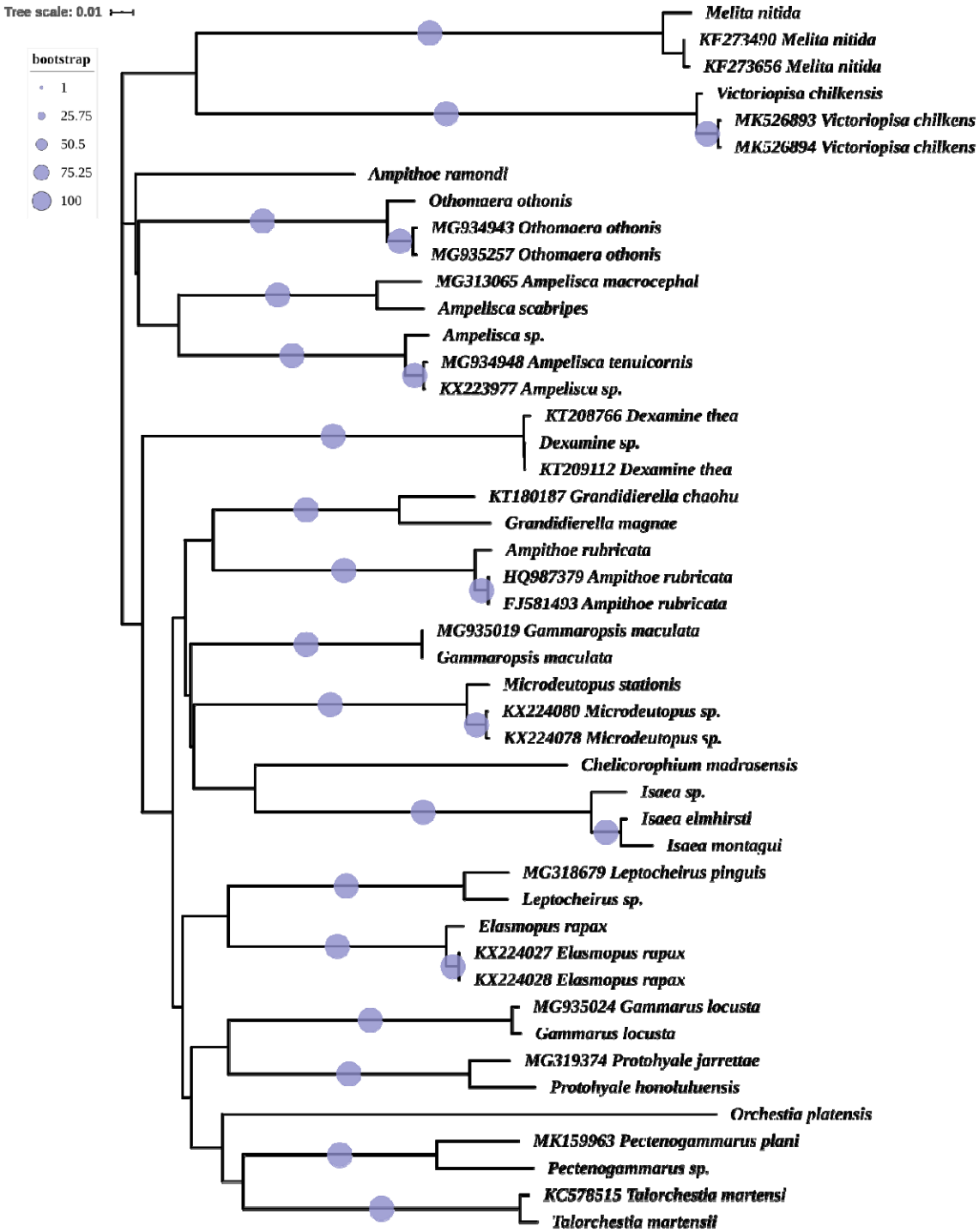

Fig. 2: NJ tree was drawn using COI sequences with Kimura-2 parametric distance model. The sequences retrieved from GenBank was represented by “accession-number_species-name”. Example; “MG935024_*Gammarus locusta*”. The sequences of the present study was represented with species name only. Time scale and bootstrap legends were given at the top left corner of the tree.

4. Conclusion

The current study provides a valuable reference library especially for those species which were barcoded for the first time, against which marine amphipods DNA barcoded from different regions can be referred in near future. Since amphipods are actively used in environmental monitoring and DNA barcoding being universal taxonomic screening tool, amphipod barcodes along with its geographic and ecological data, could not only facilitate our knowledge on taxonomy, phylogeography, and crypticism of amphipods, but also acts as a potent tool for environmental monitoring and its health assessment. It should be noted that the DNA barcoding is evolving beyond systematic or taxonomic research. The development of high-throughput sequencing technologies are significantly altering environmental surveys and bio-monitoring applications (Fonseca et al., 2010; Hajibabaei et al., 2011; Leray et al., 2015). As a result, reference datasets such as ours will become essential for assessing health and monitoring various aquatic environments using amphipod barcodes.

Acknowledgement

First author thank the Department of Science and Technology's INSPIRE fellowship (IF10431) for financial assistance.

5. References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

Antognazza CM, Britton JR, Potter C, Franklin E, Hardouin EA, Gutmann Roberts C et al (2019) Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of European anadromous shads (Alosa spp.). Aquat Conserv Mar Freshw Ecosys 29:148–152.

Bakalem, A., Hassam, N., Oulmi, Y., Martinez, M., & Dauvin, J.-C. (2020). Diversity and geographical distribution of soft-bottom macrobenthos in the bay of Bou Ismail (Algeria, Mediterranean Sea). Regional Studies in Marine Science, 33, 100938. https://doi.org/10.1016/j.rsma.2019.100938

Balasubrahmaniyan, K., & Srinivasan, M. (1987). Fouling organisms on oysters in Paravanar estuary. In Proceedings of the national seminar on estuarine management (pp. 511–513).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17, 1–17 (2016).

Bellan-Santini D (1980) Relationship between populations of amphipods and pollution. Mar Poll Bull 11:224–227

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW (2018) GenBank. Nucleic Acids Res. 2018 Jan 4; 46(D1):D41-D47.

Borowsky, B., Aitken-Ander, P., & Tanacredi, J. T. (1997). Changes in reproductive morphology and physiology observed in the amphipod crustacean, Melita nitida Smith, maintained in the laboratory on polluted estuarine sediments. Journal of Experimental Marine Biology and Ecology, 214(1–2), 85–95. https://doi.org/10.1016/s0022-0981(96)02764-5

Bousfield, E. L. (1978). A revised classification and phylogeny of amphipod crustaceans. Transactions of Royal Society of Canada Series, 4, 343–390.

Bowden, D. A., Rowden, A. A., & Attrill, M. J. (2001). Effect of patch size and in-patch location on the infaunal macroinvertebrate assemblages of Zostera marina seagrass beds. Journal of Experimental Marine Biology and Ecology, 259(2), 133–154. https://doi.org/10.1016/s0022-0981(01)00236-2

Braukmann, T. W. A. et al. Metabarcoding a diverse arthropod mock community. Mol. Ecol. Res. 19, 711–727 (2019).

Castejón-Silvo, I., Jaume, D., & Terrados, J. (2019). Feeding preferences of amphipod crustaceans Ampithoe ramondi and Gammarella fucicola for Posidonia oceanica seeds and leaves. Scientia Marina, 83(4), 349. https://doi.org/10.3989/scimar.04892.06b

Conradi M, Lopez-Gonzalez PJ, Garcia-Gomez JC (1997) The amphipod community as a bioindicator in Algeciras Bay (Southern Iberian Peninsula) based on a spatio-temporal distribution. Mar Ecol 18(2):97–111, doi:10.1111/j.1439-0485.1997.tb00430.x

Conradi, M. (2001). Relationships between environmental variables and the abundance of peracarid fauna in Algeciras bay (Southern Iberian Peninsula). Ciencias Marinas, 27(4), 481–500. https://doi.org/10.7773/cm.v27i4.504

Costa, F. O., Henzler, C. M., Lunt, D. H., Whiteley, N. M., & Rock, J. (2009). Probing marine Gammarus (Amphipoda) taxonomy with DNA barcodes. Systematics and Biodiversity, 7(4), 365–379. https://doi.org/10.1017/s1477200009990120

Cowart, D. A., Matabos, M., Brandt, M. I., Marticorena, J., & Sarrazin, J. (2020). Exploring Environmental DNA (eDNA) to Assess Biodiversity of Hard Substratum Faunal Communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and Investigate Recolonization Dynamics After an Induced Disturbance. Frontiers in Marine Science, 6. https://doi.org/10.3389/fmars.2019.00783

Cristescu, M. E. From barcoding single individuals to metabarcoding biological communities. Trends Ecol. Evol. 29, 566–571 (2014).

Deiner, K. et al. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895 (2017).

Faasse, M., van Moorsel, G. The North-American amphipods, Melita nitida Smith, 1873 and Incisocalliope aestuarius (Watling and Maurer, 1973) (Crustacea: Amphipoda: Gammaridea), introduced to the Western Scheldt estuary (The Netherlands). Aquatic Ecology 37, 13–22 (2003). https://doi.org/10.1023/A:1022120729031

Fernandez-Gonzalez J, Sanchez-Jerez P (2014) First occurrence of Caprella scaura Templeton, 1836 (Crustacea: Amphipoda) on off-coast fish farm cages in the Mediterranean Sea. Helgol Mar Res 68:187–191, doi:10.1007/s 10152-013-0375-y

Folmer O, Hoeh W, Black M, Vrijenhoek R. Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. Mol Mar Biol Biotechnol. 1994; 3:294-299. PMID: 7881515

Fonseca, V. G., Carvalho, G. R., Sung, W., Johnson, H. F., Power, D. M., Neill, S. P., … Creer, S. (2010). Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nature Communications, 1(1). https://doi.org/10.1038/ncomms1095

Franklin TW, McKelvey KS, Golding JD, Mason DH, Dysthe JC, Pilgrim KL et al (2019) Using environmental DNA methods to improve winter surveys for rare carnivores: DNA from snow and improved noninvasive techniques. Biol Cons 229:50–58.

Guerra-Garcia JM, Garcia-Gomez JC (2001) The spatial distribution of Caprellidea (Crustacea: Amphipoda): a stress bioindicator in Ceuta (North Africa, Gibraltar area). Mar Ecol PSZN 22:357–367

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A. C., & Baird, D. J. (2011). Environmental Barcoding: A Next-Generation Sequencing Approach for Biomonitoring Applications Using River Benthos. PLoS ONE, 6(4), e17497. https://doi.org/10.1371/journal.pone.0017497

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Hauton C, Tyrrell T. and Williams J (2009) The subtle effects of sea water acidification on the amphipod Gammarus locusta. 6, 1479–1489.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270, 313–321, https://doi.org/10.1098/rspb.2002.2218 (2003).

Heyde, M., Bunce, M., Wardell□Johnson, G., Fernandes, K., White, N. E., & Nevill, P. (2020). Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. Molecular Ecology Resources, 20(3). https://doi.org/10.1111/1755-0998.13148

Hodgson, A. N., Booth, A. J., David-Engelbrecht, V., & Henninger, T. O. (2014). Some life-history parameters of the non-native amphipodPlatorchestia platensis(Talitridae) in a warm temperate South African estuary. Transactions of the Royal Society of South Africa, 69(2), 97–106. https://doi.org/10.1080/0035919x.2014.941961

Horton, T. (2008). Amphipoda from marine caves of Hong Kong Island. Journal of Natural History, 42(9–12), 825–854. https://doi.org/10.1080/00222930701860124

Horton, T., Lowry, J., De Broyer, C., Bellan-Santini, D., Coleman, C. O., et al., (2019). World Amphipoda Database.<http://www.marinespecies.org/amphipoda>(accessed on 2019-02-07).

Hu, G., & Kurgan, L. (2018). Sequence Similarity Searching. Current Protocols in Protein Science, 95(1), e71. https://doi.org/10.1002/cpps.71

Hughes, L. E., & Lowry, J. K. (2010). Establishing a Neotype for Elasmopus rapax Costa, 1853 and Its Presence as an Invasive Species in Temperate Australian Waters. Journal of Crustacean Biology, 30(4), 699–709. https://doi.org/10.1651/10-3290.1

Ibabe, A., Rayón, F., Martinez, J. L., & Garcia-Vazquez, E. (2020). Environmental DNA from plastic and textile marine litter detects exotic and nuisance species nearby ports. PLOS ONE, 15(6), e0228811. https://doi.org/10.1371/journal.pone.0228811

Postma J. F., S. De Valk, M. Dubbeldam, J.L. Maas, M. Tonkes, C.A. Schipper, B.J. Kater. Confounding factors in bioassays with freshwater and marine organisms. Ecotoxicol. Environ. Saf., 53 (2002), pp. 226-237, 10.1006/eesa.2002.2195

Jażdżewska, A. M., & Mamos, T. (2019). High species richness of Northwest Pacific deep-sea amphipods revealed through DNA barcoding. Progress in Oceanography, 178, 102184. https://doi.org/10.1016/j.pocean.2019.102184

Jeunen, G., Knapp, M., Spencer, H. G., Lamare, M. D., Taylor, H. R., Stat, M., … Gemmell, N. J. (2019). Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. Molecular Ecology Resources, 19(2), 426–438. https://doi.org/10.1111/1755-0998.12982

Ji, Y. et al. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. 16, 1245–1257 (2013).

Kim, P., Yoon, T. J., & Shin, S. (2020). Environmental DNA and Specific Primers for Detecting the Invasive Species Ectopleura crocea (Hydrozoa: Anthoathecata) in Seawater Samples. Sustainability, 12(6), 2360. https://doi.org/10.3390/su12062360

Knowlton N. Sibling Species in the Sea. Annu Rev Ecol Syst. 1993; 24(1):189–216.

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Lecaudey LA, Schletterer M, Kuzovlev VV, Hahn C, Weiss SJ (2019) Fish diversity assessment in the headwaters of the Volga River using environmental DNA metabarcoding. Aquat Conserv 29(10):1785–1800

Leempoel K, Hebert T, Hadly EA (2020) A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. Proc Royal Soc B Biol Sci 287:20192353.

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proceedings of the National Academy of Sciences, 112(7), 2076–2081. https://doi.org/10.1073/pnas.1424997112

Letunic I and Bork P (2006). Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23(1):127-8

Lim J.H.C., Azam, B.A.R., Othman B.H.R. (2010) Melitoid amhipods of the genera Ceradocus Costa, 1853 and Victoriopisa Karaman and Barnard, 1979 (Crustacea: Amphipoda: Maeridae) from the South China Sea, Malaysia. 30, 23-39.

Lowry, J.K., Myers, A.A., 2017. A phylogeny and classification of the Amphipoda with the establishment of the new order Ingolfiellida (Crustacea: Peracarida). Zootaxa. https://doi.org/10.11646/zootaxa.4265.1.1.

Lyla, P. S., Velvizhi, S., & Ajmal Khan, S. (1999). List of amphipods in Parangipettai coast (Southeast coast of India). Monograph series, UGC-Special Assistance Programme, CAS in Marine Biology, Annamalai University, Parangipettai (India), 38 pp.

Martin JW, Davis GE. An updated classification of the recent Crustacea. Sci Series, 2001; 39: 1-124.

Mitchell, A. DNA barcoding demystified. Australian Journal of Entomology 47, 169–173, https://doi.org/10.1111/j.1440-6055.2008.00645.x (2008).

Mondal, N., Rajkumar, M., Sun, J., Kundu, S., Lyla, P. S., Ajmal Khan, S., & Trilles, J. P. (2010). Biodiversity of brackish water amphipods (crustacean) in two estuaries, southeast coast of India. Environmental Monitoring and Assessment, 171(1–4), 471–486. https://doi.org/10.1007/s10661-009-1292-z

Mora AJ, Prosse SWJ, Mora JA (2019) DNA metabarcoding allows non-invasive identification of arthropod prey provisioned to nestling Rufous hummingbirds (Selasphorus rufus). Peerj 7:e6596.

Myers, A. A. (1986). Amphipoda from the South Pacific: Niue Island. Journal of Natural History, 20(6), 1381–1392. https://doi.org/10.1080/00222938600770921

Naser, M., Ali, M., & White, K. (2010). Grandidierella macronyx Barnard, 1935 (Amphipoda, Aoridae): a new record from Shatt Al-Basrah, Basrah, Iraq. Crustaceana, 83(11), 1401–1407. https://doi.org/10.1163/001121610x533511

Nguyen, B.N., Shen, E.W., Seemann, J. et al. Environmental DNA survey captures patterns of fish and invertebrate diversity across a tropical seascape. Sci Rep 10, 6729 (2020). https://doi.org/10.1038/s41598-020-63565-9

Norderhaug, K., Fredriksen, S., & Nygaard, K. (2003). Trophic importance of Laminaria hyperborea to kelp forest consumers and the importance of bacterial degradation to food quality. Marine Ecology Progress Series, 255, 135–144. https://doi.org/10.3354/meps255135

Norderhaug, K.M. Use of red algae as hosts by kelp-associated amphipods. Marine Biology 144, 225–230 (2004). https://doi.org/10.1007/s00227-003-1192-7

Oh, H.-J., Krogh, P. H., Jeong, H.-G., Joo, G.-J., Kwak, I.-S., Hwang, S.-J., Gim, J.-S., Chang, K.-H., & Jo, H. (2020). Pretreatment Method for DNA Barcoding to Analyze Gut Contents of Rotifers. Applied Sciences, 10(3), 1064. https://doi.org/10.3390/app10031064

Chapman P., R. Swartz, B. Roddie, H. Phelps, P. van den Hurk, R. Butler. An international comparison of sediment toxicity tests in the North Sea. Mar. Ecol. Prog. Ser., 91 (1992), pp. 253-264, 10.3354/meps091253

Chapman P. M., F. Wang, S.S. Caeiro Assessing and managing sediment contamination in transitional waters. Environ. Int., 55 (2013), pp. 71-91, 10.1016/j.envint.2013.02.009

Parapar, J., Fernández, L., González-Gurriarán, E. & Muiño, R. 1997. Epibiosis and masking material in the spider crab Maja squinado (Decapoda: Majidae) in the Ría de Arousa (Galicia, NW Spain). Cahiers de Biologie Marine, 38(4):221-234

Persson, L.-E. (2001). Dispersal of Platorchestia platensis (Kröyer) (Amphipoda: Talitridae) along Swedish coasts: A Slow but Successful Process. Estuarine, Coastal and Shelf Science, 52(2), 201–210. https://doi.org/10.1006/ecss.2000.0735

Preissler K, Watzal AD, Vences M, Steinfartz S (2019) Detection of elusive fire salamander larvae (Salamandra salamandra) in streams via environmental DNA. Amphibia-Reptilia 40:55–64

Rabindranath, P. (1975). Marine Gammaridea (Crustacea: Amphipoda) from the Indian region; family- Ampeliscidae. Hydrobiologia, 46(2–3), 241–262. https://doi.org/10.1007/bf00043143

Radulovici AE, Archambault P, Dufresne F. DNA Barcodes for Marine Biodiversity: Moving Fast Forward? Diversity. 2010; 2(4):450–72.

Raja, S., Lyla, P. S., & Khan, S. A. (2013). Diversity of amphipods in the continental shelf sediments of southeast coast of India. Journal of the Marine Biological Association of India, 55(1), 35–41. https://doi.org/10.6024/jmbai.2013.55.1.01742.06

Ratnasingham, S., & Hebert, P. D. N. (2007). Barcoding: BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes, 7(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rattanama, K., Pattaratumrong, M. S., Towatana, P., & Wongkamhaeng, K. (2016). Three New Records of Gammarid Amphipod in Songkhla Lake, Thailand. Tropical Life Sciences Research, 27(3), 53–61. https://doi.org/10.21315/tlsr2016.27.3.8

Rehitha, T.V., Madhu, N.V., Vineetha, G. et al. Macrobenthic fauna with special reference to the ecology and population structure of a tubicolous amphipod, Chelicorophium madrasensis (Nayar, 1950) in a tropical estuary, southwest coast of India. Mar Biodiv 49, 1013–1026 (2019). https://doi.org/10.1007/s12526-018-0886-5

Reinhardt T, van Schingen M, Windisch HS, Nguyen TQ, Ziegler T, Fink P (2019) Monitoring a loss: Detection of the semi-aquatic crocodile lizard (Shinisaurus crocodilurus) in inaccessible habitats via environmental DNA. Aquat Conserv 29:353–360

Rym Zakhama-Sraieb, Intissar Mnasser, Imen Zribi & Faouzia Charfi-Cheikhrouha (2017) Update of checklist of marine Amphipoda in Tunisia from 2009 to April 2017. Biodiversity Journal, 8 (2): 493–496

Sales NG, Wangensteen OS, Carvalho DC, Deiner K, Præbel K, Coscia I et al (2020) Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. BioRxiv. https://doi.org/10. 1101/2020.02.04.933366

Sampaio, L., Mamede, R., Ricardo, F., Magalhães, L., Rocha, H., Martins, R., Dauvin, J.-C., Rodrigues, A. M., & Quintino, V. (2016). Soft-sediment crustacean diversity and distribution along the Portuguese continental shelf. Journal of Marine Systems, 163, 43–60. https://doi.org/10.1016/j.jmarsys.2016.06.011

Sanchez-Jerez P, Barberà-Cebriàn C, Ramos-Espla AA (1999) Comparison of the epifauna spatial distribution in Posidonia oceanica, Cymodocea nodosa and unvegetated bottoms: importance of meadow edges. Acta Oecolog 20: 391–405. doi:org/10.1016/S1146-609X(99)00128-9

Sansom BJ, Sassoubre LM (2017) Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. Environ Sci Technol 51:14244–14253

Sawaya, N. A., Djurhuus, A., Closek, C. J., Hepner, M., Olesin, E., Visser, L., … Breitbart, M. (2019). Assessing eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through environmental DNA metabarcoding. Ecology and Evolution, 9(3), 1029–1040. https://doi.org/10.1002/ece3.4742

Seetharaman, K., & Kandasamy, K. (2011). Reproductive biology of a natural mangrove hybrid Rhizophora annamalayana and its parent species (R. apiculata and R. mucronata) (Rhizophoraceae). Botanica Marina, 54(6). https://doi.org/10.1515/bot.2011.062

Shelton AO, Kelly RP, O'Donnell JL, Park L, Schwenke P, Greene C et al (2019) Environmental DNA provides quantitative estimates of a threatened salmon species. Biol Conserv 237:383–391.

Srinivas, T., Sukumaran, S., Mulik, J., & Dias, H. Q. (2019). Community structure of benthic amphipods in four estuaries of northwest India. Regional Studies in Marine Science, 27, 100532. https://doi.org/10.1016/j.rsma.2019.100532

Stoeckle, M. Y., Das Mishu, M., & Charlop-Powers, Z. (2020). Improved Environmental DNA Reference Library Detects Overlooked Marine Fishes in New Jersey, United States. Frontiers in Marine Science, 7. https://doi.org/10.3389/fmars.2020.00226

Sutter M, Kinziger AP (2019) Rangewide tidewater goby occupancy survey using environmental DNA. Conserv Genet 20:597–613

Taberlet, P. et al. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Mol. Ecol. 21, 1816–20 (2012a).

Taberlet, P., Coissac, E., Hajibabaei, M. & Rieseberg, L. H. Environmental DNA. Mol. Ecol. 21, 1789–93 (2012b).

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z (2012) Estimation of fish biomass using environmental DNA. PLoS ONE 7:e35868

Tempestini, A., Rysgaard, S., & Dufresne, F. (2018). Species identification and connectivity of marine amphipods in Canada's three oceans. PLOS ONE, 13(5), e0197174. https://doi.org/10.1371/journal.pone.0197174

Thompson,J.D., Gibson,T.J., Plewniak,F., Jeanmougin,F. and Higgins,D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 25:4876-4882.

Thomsen PF, Willerslev E (2015) Environmental DNA—an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18.

Ugolini, A. The moon orientation of the equatorial sandhopper Talorchestia martensii Weber. Behav Ecol Sociobiol 70, 1699–1706 (2016). https://doi.org/10.1007/s00265-016-2175-2

Ugolini, A., & Ciofini, A. (2015). Landscape vision and zonal orientation in the Equatorial sandhopper Talorchestia martensii. Journal of Comparative Physiology A, 202(1), 1–6. https://doi.org/10.1007/s00359-015-1047-y

Vinogradov ME, Causey D, Semenova TN, Volko AF. Hyperiid amphipods (Amphipoda, Hyperiidea) of the world oceans. Smithsonian Institution, Washington, DC. 1996

Virnstein RW (1987) Seagrass-associated invertebrate communities of the southeastern USA: a review. Fla Mar Res Publs 42:89–116

Wongkamhaeng, K., Nabhitabhata, J., & Towatana, P. (2015). Corophiine amphipods of the genera Chelicorophium and Paracorophium from the lower Gulf of Thailand (Crustacea,

Amphipoda, Corophiidae, Corophiinae). ZooKeys, 505, 35–50. https://doi.org/10.3897/zookeys.505.9751

Yokoyma, H., Higano, J., Adachi, K., Ishiht, Y., & Yamada, Y. (2002). Evaluation of shrimp polyculture system in Thailand based on stable carbon and nitrogen isotope ratios. Fisheries Science, 68(4), 745–750. https://doi.org/10.1046/j.1444-2906.2002.00488.x

Zakhama-Sraieb R, Sghaier YR, Charfi-Cheikhrouha F (2006) Is amphipod diversity related to the quality of Posidonia oceanica beds? Biol Mar Medit 13:174–180

Zanders, I.P., Rojas, W.E. Cadmium accumulation, LC50 and oxygen consumption in the tropical marine amphipod Elasmopus rapax. Marine Biology 113, 409–413 (1992). https://doi.org/10.1007/BF00349166

Zhang Y, Pavlovska M, Stoica E, Prekrasna I, Yang J, Slobodnik J et al (2020b) Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to marine mammals. Environ Int 135:105307