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Genetic evidences of multiple phyletic lineages of *Capitella capitata* (Fabricius 1780) complex in the Mediterranean Region

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Abstract

In the present work we provide preliminary genetic evidences of several phyletic lineages of *Capitella capitata* (Fabricius 1780) in the Mediterranean Region. A portion of taxonomic informative nucleotide sequence (COI) was investigated in *C. capitata* samples from five different sites along the Italian coasts. Sequences analysis carried out with both Kimura genetic distances and Bayesian method support the existence of five clusters. According to the 10 times fold method, widely applied for DNA Barcoding, the 5 clusters identified can be considered different provisional species, one for each sampling site. Given the importance of *C. capitata* as indicator organism for polluted marine environments, and considering that different species or lineages may show different tolerance to pollutants, a deeper knowledge on the number and distribution of existing *C. capitata* species should be pursued.

Introduction

The Annelida Polychaeta taxa formerly described as *Capitella capitata* (Fabricius 1780) consists of numerous sibling species characterized by a short life cycle with high potential for population growth and high speciation and extinction rates (Chareonpanich et al. 1994; Cognetti & Maltagliati 2000). Considered a highly opportunistic taxa, *C. capitata* is widely used as indicator for organically polluted and disturbed marine environments (Grassle & Grassle 1974; Pearson & Rosenberg 1978; Tsutsumi 1987; Tsutsumi et al. 1990; Chang et al. 1992; Linke-Gamenick et al. 1998; Blake et al. 2009; Tomassetti et al. 2009; Tomassetti et al. 2016). First evidences for the existence of *C. capitata* sibling species were based on enzymatic polymorphisms (Grassle & Grassle 1976) and karyotypes (Grassle et al. 1987). The six species identified by Grassle & Grassle (1976) from North American samples showed similar morphology, distinct life histories and different reproductive modes. The high morphological uniformity among *C. capitata* spp. has probably hampered so far a thorough taxonomic revision of this taxa. Recently, a redescription of the species *C. capitata* on a neotype from Greenland was made by Blake (2009). Several studies, carried out on both North American and European lineages, demonstrated that different species of *C. capitata* spp. show different tolerance to pollutants and to disturbed environments due to species specific life-history traits and physiological adaptations (Linke-Gamenick et al. 1998, 2000; Mendez et al. 2001). Although morpho-

logically similar, *C. capitata* species show genetically fixed ecophysiological differences, most probably driven by the abiotic stress conditions, with the result that some species may be considered more opportunistic than others. Given the wide use of *C. capitata* as indicator of polluted environments, knowledge on the number and distribution of existing species is worth to be investigated. A standardized tool for taxonomic identification is the DNA barcode, based on partial nucleotide sequence (≅650bp) of mitochondrial COI gene (Hebert et al. 2003, Savolainen et al. 2005, Ward et al. 2005, Radulovici et al. 2010, Taberlet et al. 2012). So far, the records of *C. capitata* barcoded and published in the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) are mainly from Canada and India, and assigned to two different provisional species through the Barcode Index Number (BIN) system (Carr et al. 2011, Ratnasingham & Hebert 2013). In this work the DNA barcode of *C. capitata* from Mediterranean region was investigated for the first time providing preliminary evidences for the existence of several phyletic lineages.

Materials and Methods

Specimens of *C. capitata* were obtained from different sampling campaigns along the Italian coasts and preserved either in formalin or in alchool (Tab.1). The specimens were identified by the following diagnostic morphological characters: setal arrangement in thoracic segments, prostomium shape, numbers of genital spines in the male, shape and dorsal cleft in the pygidium (Claparède 1870; Eisig 1887; Fauvel 1927; Hartman 1947; Day 1967; Gravina & Somaschini 1990; Blake 2000; Blake 2009; Blake et al. 2009; Garcia-Garza et al. 2011). The methyl green staining protocol was used as a taxonomic method to highlight diagnostic characters (Winsnes 1985) (Figure 1). A total of 20 specimens, 4 for each sampling site, were used for genetic analysis. Prior to DNA extraction, samples were kept in standard phosphate-buffered saline solution for 1 h. Silica gel spin columns (Promega; www.promega.com) were used to isolate total DNA from 30 mg of tissue. The set of primers LCO1490/ HC02198 was used to amplify partial sequences of the mitochondrial COI gene. The polymerase chain reaction (PCR) products, previously purified on silica gel spin columns (Promega; www.promega.com) were directly sequenced on an ABI 3730 automatic sequencer (Applied Biosystems; www.appliedbiosystems.com) using the same PCR primers. Universal DNA primers and PCR conditions were derived from Folmer et al. (1994). The obtained sequences were aligned with ClustalW (Thompson et al. 1994). Kimura genetic distances among sampling sites and Bootstrap test of phylogeny based on minimum evolutionary network among haplotypes were assessed with the software MEGA 3.1 (Kumar et al. 2004). Phylogenetic reconstructions of haplotypes was also performed by Bayesian method implemented in Mr Bayes 3.1 (Ronquist and Huelsenbeck 2003) using the general time reversible evolutionary model with gamma-distributed rate variation across sites and a proportion of invariable sites using the default priors. A total of 21 nucleotide sequences of partial COI mitochondrial gene available online were retrieved and used in the analysis: 18 sequences of *C. capitata* cmplx, 2 sequences of Polycheata and 1 sequence of *Barantolla americana,* used as outgroup, were retrieved from the BOLD database. The software BOLD Identification System (IDS) (Ratnasingham and Hebert 2007) was used to check whether the sequences obtained in the present work matched to reference barcodes in BOLD database.

Table 1. Specimens sampling information

Locations	Sample Code	Geographic coordinates	Sampling gear	Fixation	Water depth "m"	Biotope
Bisceglie (Barletta-Andria-Trani)	CC_BI	41°15,021'N 16°32,734'E	Van Veen grab	Formalin	22	Marine fish farm
Porto Ercole (Grosseto)	CC_PE	42°23,250'N 11°14,573'E	Van Veen grab	Formalin	26	Marine fish farm
Orbetello (Grosseto)	CC_OB	42°25,968'N 11°09,724'E	Manual collection	Formalin	1	Land based fish farm (lagoon)
Follonica (Grosseto)	CC_FP	42°54,858'N 10°38,545'E	Manual collection	Alcohol	1	River mouth
Sabaudia (Latina)	CC_LS	41°16,149'N 13°02,685'E	Van Veen grab	Formalin	2	Coastal lake

Figure 1 Methyl green staining reaction of anterior female of CC_OB sample.

Results

All the specimens investigated in this study were morphologically indistinguishable and classified as *C. capitata*: they all showed conical and rounded prostomium, peristomium with a single asetigerous ring, thorax with nine segments, capillary setae in neuropodia of setigers 1-9 and eyes absent, in the male genital spines of setigers 8 visible externally, pygidium with a single lobe; besides, the methyl green staining reaction showed a *C. capitata* staining pattern (Blake 2009) (Figure 1). The number of specimens successfully sequenced *vs* the number of processed specimens were 1:4, imputable to formalin preservation. A total of 7 good quality sequences were obtained from 5 different sampling sites (GenBank accession numbers KU050696-KU050702). All sequences could be translated in portions of functional proteins, final sequence alignment was 676 bp long and showed the following average nucleotide composition T 40.5%, C 17.5%, A 21.6%, G 20.4%; 209 variable sites, 155 parsimony informative sites and 54 singleton sites.

None of the obtained sequences matched to any species or BIN in BOLD database, however, when the search was made including all barcode records on BOLD (both private and published sequences), the Mediterranean samples fell in the ample group of sequences named *Capitella capitata* and *Capitella* sp. Most of the sequences that clustered with the Mediterranean samples are private, except two published Polycheata sequences WCH_0756 and WCH_0841, which were retrieved and included in the data analysis. The similarity values produced by the BOLD Identification System (IDS) are the following: 99.83%-98.97% between CC_OA and *Capitella capitata* private sequences from China Shandong; 97.18%-96.55% between CC_PE and *Capitella capitata* private sequences from Norway; 96.28%-95.58% between CC_BI and Polychaeta published sequences from US Virginia; 89.15%-88.84% between CC_FP and *Capitella* sp. B private sequences from Barazil Rio de Janeiro; 84.47%-84.3% between CC_LS and *Capitella* sp. B private sequences from Barazil Rio de Janeiro.

The minimum evolutionary network, based on Kimura 2 parameters distances, and the phylogenetic tree, obtained with the Baysian method (Figure 2), are characterized by 7 distinct phyletic lineages of *C. capitata* complex. Two of these lineages are represented by the two BINs described in the BOLD database (AAE7885 and AAE7888), whereas the other five are represented by the 5 Mediterranean sampling sites specimens; the two Polycheata sequences cluster wich the Mediterranean sample CC_BI. The identified clusters appear as independent phyletic lineages on the tree, with the exception of the two sampling sites CC_LS and CC_FP which show a posterior probability of 99 with the Baysian method. The Net genetic distances based on Kimura 2 parameter method among

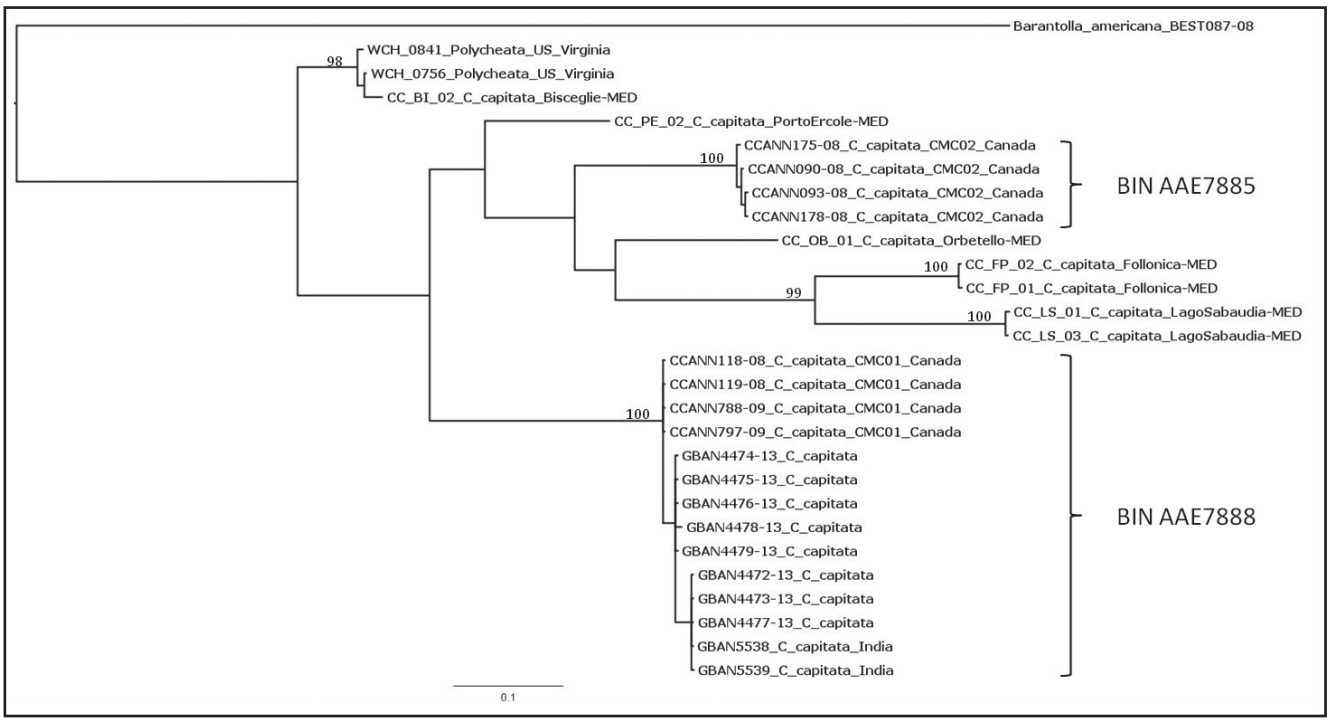

Figure 2 Phylogram of *C capitata* haplotypes of mitochondrial COI partial gene obtained with Bayesian method. Split posterior probability > 95 is reported. Sequences retrieved from BOLD System are named with BOLD sequence ID and are represented by 18 sequences of *C. capitata* spp. belonging to two different BINs: AAE7885 and AAE7888, by 2 sequences of Polycheata (WCH_0756; WCH_0841) and by the sequence of *B. Americana* (BEST087-08) used as outgroup. The sequences obtained in the present work from Mediterranean samples are: CC_BI_02, CC_FP_01, CC_FP_02, CC_LS_01, CC_LS_03, CC_OB_01, CC_PE_02.

the 8 groups of sequences (7 lineages of *C. capitata* complex plus the outgroup *B. americana*), and the average intra-lineage distances calculated for lineages with more than 1 specimens are reported in Tab. 2. The average inter-lineage distance among *C. capitata* groups is 20.7% ranging between 15.41% CC_OA – CC_BI and 25.08% CC_PE – CC_FP.

Discussion

Several studies have been carried out on *C. capitata* in relation to life history traits and environmental ecology, also due to its use as biological indicator, however, little effort has so far been put into investigation of its taxonomic status. Besides, the identification of *C.*

Table 2. Net genetic distances based on Kimura 2 parameter method among *C. capitata* lineages and average intra-lineage distances calculated for lineages with more than 1 specimen.

	BIN AAE7888	BIN AAE7885	CC_BI	CC_OA	CC_PE	CC_FP	CC_LS	OutGr
BIN AAE7888	0,0111							
BIN AAE7885	0,2189	0,0013						
CC_BI	0,1986	0,2164	0,015					
CC_OA	0,2003	0,1655	0,1747	___				
CC_PE	0,1787	0,1868	0,1541	0,1898	___			
CC_FP	0,2395	0,2396	0,2421	0,2175	0,2508	0,00		
CC_LS	0,2039	0,2346	0,2167	0,2327	0,2007	0,1801	0,00	
OutGr	0,4402	0,4109	0,4312	0,4452	0,4466	0,4278	0,4430	___

capitata lineages, so far based on diverse parameters (i.e. ecophysiological characteristics, karyotypes and enzyme patterns, ultrastructure of eggs and ovarian follicle cells) has not been supported by nucleotide sequence data. The great difficulty in distinguishing *C. capitata* species by morphological characters (Blake et al. 2009) should encourage the use of molecular barcoding on this polychaeta. The results obtained in this study indicate the presence of multiple phyletic lineages of *C. capitata* in the Italian Seas. According to the 10 times fold method, widely applied for DNA Barcoding (Hebert et al. 2004, Witt et al. 2006), the 5 clusters identified can be considered different provisional species of *C. capitata*, one for each sampling site. The different lineages show no genetic relationship among them, the only exception is given by CC_LS and CC_FP which also share a similar habitat associated to salinity variations of coastal lagoon and river mouth respectively.

Though it is known that *C. capitata* is a complex of species, such a high diversity was not expected. So far, barcoding data on *C. capitata* support the existence of 2 provisional species (BINs), one of which including samples from geographically distant sites such as Canada and India. Besides, if we assume that a similarity value > 95% between nucleotide COI sequences of *C. capitata* is indicative of conspecific individuals, the results obtained with the BOLD Identification System (IDS) suggest that *C. capitata* species found in the Italian Seas have large geographic distribution, including very distant areas such as China, Norway and US. Cryptic species complexes are not uncommon in invertebrates, and several cases have been reported for polychaetes (Nigren 2014). However, *C. capitata* seems to harbor an exceptional number of lineages, probably due to ecological and demographic factors; the high tolerance to polluted areas favor rapid demographic explosions that may accelerate the evolutionary process due to genetic drift. Moreover, the diverse habitats occupied by *Capitella* spp. in terms of water depth, salinity and most importantly organic enrichment level, could account for adaptive speciation associated to ecological segregation.

One relevant aspect about *C. capitata* species complex is that the different lineages may show different level of tolerance to pollution (Linke-Gamenick et al. 1998, 2000; Mendez et al. 2001). This point makes much more important the development of a straightforward and low cost method for species identification. The high number of provisional species found in a rather delimited geographic area suggests the existence of many species of *C. capitata* in the Mediterranean Sea still to be uncovered.

References

Blake J.A. 2000. Family Capitellidae Grube, 1862 In: Blake JA, Hilbig B, Scott PV (Eds) Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and the Western Santa Barbara Channel. Volumen 7. The Annelida Part 4, Polychaeta: Flabelligeridae to Sternaspidae: Santa Barbara Museum of Natural History, California, 47-96.

Blake J.A. 2009. Redescription of *Capitella capitata* (Fabricius) from West Greenland and designation of a neotype (Polychaeta, Capitellidae). Zoosymposia 2:55-80.

Blake J.A., Grassle J.P., Eckelbarger J.K. 2009. *Capitella teleta*, a new species designation for the opportunistic and experimental *Capitella* sp. I, with a review of the literature for confirmed records. Zoosymposia 2:25-53.

Carr C.M., Hardy S.M., Brown T., Sheldon T., Macdonald T., Hebert P.D.N. 2011. A Tri-Oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian Polychaetes. PLoS ONE 6:e22232. 12 pages.

Chang S., Steimle F.W., Reid R.N., Fromm S.A., Zdanowicz V.S., Pikanowski R.A. 1992. Association of benthic macrofauna with habitat types and quality in the New York Bight. Marine Ecology Progress Series 89:251-253.

Chareonpanich C., Montani S., Tsusumi H., Nakamura H.. 1994. Estimation of oxygen consumption of a deposit feeding polychaete *Capitella* sp. I. Fisheries Science 60:249-251.

Claparède E. 1870. Les Annélides Chétopodes du Golfe de Naples. Memories de la Societé Physique de Genéve 20:1-225.

Cognetti G., Maltagliati F. 2000. Biodiversity and adaptive mechanisms in brackish water fauna. Marine Pollution Bulletin 40(1):7-14.

Day J. 1967. A monograph on the Polychaeta of Southern Africa. British Museum of Natural History Publications 656:1-878.

Eisig H. 1887. Monographie der Capitelliden des Golfes von Neapel. Fauna und Flora des Golfes von Neapel, 16:1-906.

Fabricius O. 1780. Fauna Groenlandica. Hafniae et Lipsiae xiv + 452 pp.

Fauvel P. 1927. Polychètes Sedentaires & Addenda aux Poly-

chètes Errantes. Faune de France. Fédération Française des Sociétés de Science Naturel 16:1-494.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:194-299.

Garcia-Garza M.E., De Leon-Gonzalez J.A. 2011. Review of the Capitellidae (Annelida, Polychaeta) from the Eastern Tropical Pacific region, with notes on selected species. Zookeys 151:17-52.

Grassle J.P., Gelfman C.E., Mills S.W. 1987. Karyotypes of *Capitella* sibling species and of several species in the related genera Capitellides and Capitomastus (Polychaeta). Bulletin of the Biological Society of Washington 7:77–88.

Grassle J.F., Grassle J.P. 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes. Journal of Marine Research 32:253-284.

Grassle J.P., Grassle J.F. 1976. Sibling species in the marine pollution indicator *Capitella* (Polychaeta). Science 192:567-569.

Gravina M.F., Somaschini A. 1990. Censimento dei policheti dei mari italiani: Capitellidae Grube, 1862. Atti Società Toscana di Scienze Naturali, Memorie Serie B 97I:259-285.

Hartman O. 1947. Polychaetous annelids Part VII. Capitellidae. Allan Hancock Pacific Expeditions, California 10:391-481.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B 270:313-321.

Hebert P.D.N., Stoeckle M.Y., Zemlak T.S., Francis C.M. 2004. Identifications of birds through DNA barcodes. PLoS Biology 2:1657-1663.

Kumar S., Tamura K., Nei M. 2004. MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5:150-163.

Linke-Gamenick I., Vismann B., Grieshaber M.I., Giere O. 1998. Ecophysiological differentiation of *Capitella capitata* (Polychaeta). Sibling species from different sulfidic habitats. Marine Ecology Progress Series 175:155-166.

Linke-Gamenick I., Forbes V.E., Méndez N. 2000. Effects of chronic fluoranthene exposure on sibling species of *Capitella* with different development modes. Marine Ecology Progress Series 203:191-203.

Méndez N., Linke-Gamenick I., Forbes V.E., Baird D.J. 2001. Sediment processing in *Capitella* spp. (Polychaeta: Capitellidae): strain-specific differences and effects of the organic toxicant fluoranthene. Marine Biology 138:311-319.

Nigren A. 2014. Cryptic polychaete diversity: a review. Zoologica Scripta 43(2):172-183.

Pearson T.H., Rosemberg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology 16:229-311.

Radulovici A.E., Archambault P., Dufresne F. 2010. DNA Barcodes for Marine Biodiversity: Moving Fast Forward? Diversity 2:450-472.

Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7:355-364.

Ratnasingham S., Hebert P.D.N. 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(8):e66213. 16 pages.

Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Savolainen V., Cowan R.S., Vogler A.P., Roderick G.K., Lane R. 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 360:1805-1811.

Taberlet P., Coissac E., Pompanon F., Brochmann C., Willerslev E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21:2045-2050.

Thompson J.D., Higgins D.G., Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Tomassetti P., Persia E., Mercatali I., Vani D., Marusso V., Porrello S. 2009. Effects of mariculture on macrobenthic assemblages in a Western Mediterranean site. Marine Pollution Bulletin 58:533-541.

Tomassetti P., Gennaro P., Lattanzi L., Mercatali I., Persia E., Vani D., Porrello S. 2016. Benthic community response to sediment organic enrichment by Mediterranean fish farms: case studies. Aquaculture 450:262-272.

Tsutsumi H. 1987. Population dynamics of *Capitella capitata* (Polychaeta, Capitellidae) in an organic polluted grove. Marine Ecology Progress Series 36:139-149.

Tsutsumi H., Fukunaga S., Fujita N., Sumida M. 1990. Relationship between growth of *Capitella* sp. and organic enrichment of the sediment. Marine Ecology Progress Series 63:157-162.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N. 2005. DNA barcoding Australia's fish species. Philosophical transactions of the Royal Society of London.

Series B, Biological sciences 360:1847-1857.

Winsnes I.M. 1985. The use of Methyl Green as an aid in species discrimination in Onuphidae (Annelida, Polychaeta). Zoologica Scripta 14(1): 19-23.

Witt J.D.S., Threloff D.L., Hebert P.D.N. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: Implications for desert spring conservation. Molecular Ecology 15:3073-3082.