

Mediterranean Marine Science

Vol 16, No 3 (2015)



Morphological and genetic barcoding study confirming the first *Stegastes variabilis* (Castelnaud, 1855) report in the Mediterranean Sea

A. VELLA, S. AGIUS DARMANIN, N. VELLA

doi: [10.12681/mms.1391](https://doi.org/10.12681/mms.1391)

To cite this article:

VELLA, A., AGIUS DARMANIN, S., & VELLA, N. (2015). Morphological and genetic barcoding study confirming the first *Stegastes variabilis* (Castelnaud, 1855) report in the Mediterranean Sea. *Mediterranean Marine Science*, 16(3), 609–612. <https://doi.org/10.12681/mms.1391>

Morphological and genetic barcoding study confirming the first *Stegastes variabilis* (Castelnaud, 1855) report in the Mediterranean Sea

A. VELLA, S. AGIUS DARMANIN and N. VELLA

Conservation Biology Research Group, Department of Biology, University of Malta, Msida MSD2080, Malta

Corresponding author: adriana.vella@um.edu.mt

Handling Editor: Argyro Zenetos

Received: 13 June 2015; Accepted: 13 July 2015; Published on line: 7 October 2015

Abstract

This paper presents morphometric and genetic barcoding analyses of the first record of the Cocoa Damselfish, *Stegastes variabilis* in the Mediterranean Sea. A single specimen was captured from Senglea waterfront, Malta (Central Mediterranean) on the 15th of September 2013. The species is non-indigenous in the Mediterranean, as it is native to the tropical Western Atlantic. Apart from undertaking identification through meristics and morphometric measurements of the specimen, genetic analyses of the 3410 bp mtDNA genes were carried out to confirm the species' identity. The latter was useful given that the genus *Stegastes* is known to be composed of morphologically very similar species, with variable colour patterns.

Keywords: *Stegastes variabilis*, alien species, genetic barcoding, fish morphometrics, Mediterranean Sea, Malta

Introduction

Pomacentridae is an ecologically important species-rich and highly abundant Family of reef fishes (Brunt & Davies, 1994) composed of more than 350 species that primarily inhabit tropical and temperate near-shore, shallow waters around the world (Allen & Woods, 1980; Bessa *et al.*, 2007; Cooper, *et al.*, 2009; Feitosa *et al.*, 2012; Litsios, *et al.*, 2012; Froese & Pauly, 2015), with a preference for hard rocky or reef substrates (Bessa *et al.*, 2007). Some species reach a maximum length of 35 centimetres, although most species are usually less than 15 centimetres (Carpenter, 2002; Froese & Pauly, 2015).

Damselfish species in the Mediterranean are represented by the native *Chromis chromis* (Linnaeus, 1758) which is distributed from the eastern Atlantic along the coast of Portugal to the Gulf of Guinea and the Mediterranean Sea (Dulčić, 2005; Froese & Pauly, 2015).

Introduced Pomacentridae species of the genus *Abudefduf* (Linnaeus, 1758) (Goren & Galil 1998; Azzurro *et al.*, 2013; Tsadok *et al.* 2015) have been reported in the Mediterranean in 1957 from the Gulf Naples in Italy (Tardent, 1959), where a young individual was identified as *Abudefduf saxatilis vaigiensis*. Western Mediterranean records followed by another from the Gulf of Genoa in the Ligurian Sea (Vacchi & Chiantore 2000). However there have also been reports of species of this genus entering the Mediterranean Sea through the Suez Canal with a first recorded in Israel in 1998 (Golani *et al.*, 2012), while other reports of this genus have been recorded more recently along the coast of Tarragona in Spain (Azzurro *et al.*, 2013) and in Malta by the authors in 2013 (Vella, 2014 a & b), followed by Deidun & Cas-

triota (2014). These local reports were in turn listed in a review by Evans *et al.* (2015). Another Pomacentridae that has been recorded in the Mediterranean, *Chrysiptera cyanea* (Quoy & Gaimard, 1825), was collected from the Gulf of Trieste, North Adriatic Sea (Lipej *et al.*, 2014).

Methods

On the 15th of September, 2013 during ongoing research by the Conservation Biology Research Group of the University of Malta (CBRG-UoM) in collaboration with Maltese sport fishermen, a single individual (Fig. 1) of total body length 113.4 mm was captured during a shore sport-fishing competition along the Senglea waterfront (GPS: 35.891074N, 14.514230E), Malta. It was caught using a reel rod with paternoster rig set-up using a 0.16 mm line and two hooks size 10, and *Alitta virens* (M. Sars, 1835) as bait placed at a depth of 1 to 2 metres, around 0.5 to 1 metres from the seabed. Upon capture the whole specimen was kept for scientific analyses. The diagnostic features that were used in the morphological identification of the specimen were analysed under a 20X magnification dissecting microscope. All length measurements were taken to the nearest 0.1 mm using electronic calipers and mass was recorded to the nearest 0.1 g.

A small piece of muscle tissue was stored in 96% ethanol, later digested with Proteinase K and the total DNA was extracted using AccuPrep[®] Genomic DNA Extraction Kit (Bioneer). PCR amplifications was carried out for the complete sequence of cytochrome b (cytb) using GluDG-L14724, CB3H-15560 and CB6ThrH-15930 primers (Martin & Palumbi, 1993) and NADH dehydro-

genase subunit 3 (nd3) using nd3-F270 and nd3-R750 primers (Cooper *et al.*, 2009). The partial sequence of cytochrome c oxidase I gene (COI) using FishF2 and FishR2 primers (Ward *et al.*, 2005) and 12S to 16S ribosomal RNA genes (12S-16S) using 12SA and 16SA primers (Palumbi, 1996) were also amplified following the protocols of their respective sources. PCR products were sequenced using Applied Biosystems 3730xl. The sequences obtained were deposited in Genbank with accession numbers: KT002410 (cytb); KT002411 (nd3); KT002412 (COI); and KT002413 (12S to 16S). These sequences were compared to other sequences available in GenBank using BLASTn.

Results

Morphological analyses

The fish specimen's morphology, meristics (Table 1) and colour (Fig. 1), matched with the descriptions of the species *Stegastes variabilis* given by Allen & Woods (1980), Allen (1991), McEachran & Fechhelm (2010) and Froese & Pauly (2015). The specimen had XII+15 dorsal finrays, II+12 anal finrays and 1+5 ventral finrays. The body had a dark yellow to brownish colouration (Figure 1 and Table 1) similar to one recorded by Souza *et al.* (2011).

This specimen lacked the large black spot on the rayed section of the dorsal fin, possibly because the occurrence of this spot is mostly visible in juveniles (Allen, 1991). Moreover Souza *et al.*, (2011) has noted that *S. variabilis* exhibits a wide range of colouration and did not exclude the possibility of an undescribed species. For this purpose, a molecular genetics approach was considered to confirm the species' identification.

Genetic analyses

A total of 3410bp were sequenced, 543 bp, 1140 bp, 351 bp and 1376 bp obtained from COI, cytb, nd3 and 12S-16S respectively, and each were run via blastn to identify sequence matches. The cytb and nd3 gene sequences confirmed the genus, as the closest identity matches (78.1% to 92.6% and 80.0% to 98.0% respectively) were with those of the genus *Stegastes*. The 543 bp of COI sequence resulted in 100% identity match to *S. variabilis* [gb|JQ842713; JQ842714; JQ841972 (Weigt *et al.*, 2012)]. Additionally, the 308 bp portion of the 12S rRNA gene sequence also gave a 100% identity match to *S. variabilis* [gb|AF285938 (Tang, 2001)], confirming the species identification of the specimen. The latter forms part of the CBRG-UoM marine specimen and tissues collection with the reference number: CBRG/F.130915/SV001.

Discussion

These results provide relevant scientific data related to the first record of *S. variabilis* in the Maltese waters

and the Mediterranean Sea. These results also confirm that this first documented occurrence of *S. variabilis* in the Mediterranean Sea reported by Vella (2014 a & 2014 b) and subsequently listed in Evans *et al.*'s (2015) review as 'questionable' is in fact certain and confirmed as shown by the results presented here.

Most of the alien fishes recorded in the Mediterranean are considered to be Lessepsian migrants (Golani, 2010) which entered through the Suez Canal, followed by adaptation in the Levantine Sea before spreading westerly throughout the Mediterranean Sea (Psomadakis *et al.*, 2009; Jribi & Bradai, 2012; Souissi *et al.*, 2014; Evans *et al.* 2015). As *S. variabilis* has never been recorded in any other region of the Mediterranean Sea its occurrence in the Southern Central Mediterranean indicates that this specimen may have not been spotted before due to its close resemblance to the Mediterranean damselfish, *C. chromis*. Moreover, the natural habitat of *S. variabilis* consists of inshore and offshore algal and rocky reefs, usually at 0 to 30 metres (Allen, 1991; McEachran & Fechhelm, 2010), a habitat similar to that occupied by *C. chromis*. These two factors make it difficult for the occurrence of this alien species to be detected.

This alien species could have been carried from its natural habitat range (Western Atlantic) by vessel transportation, such as in ship ballast water tanks (Galil, 2006) given that the specimen was found in a port which is regularly visited by large yachts, cruise-liners and other commercial ships. Another possibility is that the specimen was transported for the aquarium trade given that this species is also reported as an ornamental species both outside and within the Mediterranean (Monteiro-Neto *et al.*, 2003; Pappavaslopoulou *et al.*, 2014), similarly to the above mentioned *C. cyanea* (Lipej *et al.*, 2014).

The occurrence of *S. variabilis* in the Mediterranean Sea must be closely traced, as the damselfish of the genus



Fig. 1: The individual *Stegastes variabilis* caught from Senglea Waterfront, Malta on the 15th of September 2013 (image by S. Agius Darmanin, CBRG-UoM).

Table 1. Measurements and meristics for *Stegastes variabilis* specimen caught in Malta.

Measurements	mm	Proportion %
Total length (TL)	113.4	
Fork length (FL)	105.9	93.39 TL
Standard length (SL)	83.0	73.19 TL
Maximum body depth (BD)	46.7	41.18 TL
Length of dorsal fin base	56.7	68.31 SL
Pectoral fin base	4.5	5.42 SL
Anal fin base	21.1	25.42 SL
Pre-pelvic length	31.6	38.07 SL
Pre-anal length	61.6	74.21 SL
Head length (HL)	28.2	24.87 TL
Pre-orbital length	7.3	25.89 HL
Eye diameter	6.9	6.08 TL
Counts		
Dorsal fin spines	12	
Dorsal fin soft rays	15	
Ventral fin spines	1	
Ventral fin soft rays	5	
Anal fin spines	2	
Anal fin soft rays	12	

Stegastes are considered to be among the most aggressive and highly territorial benthic feeders (Letourneur *et al.*, 1997; Santangelo *et al.*, 2002; Osório *et al.*, 2006; Hutchings *et al.*, 2008; Steele *et al.*, 2010), and show to have significant impact on coral community structure and algal assemblages (Gobler *et al.*, 2006), increasing the algal biomass and productivity of reef systems (Hinds & Ballantine, 1987; Ferreira *et al.*, 1998; Ceccarelli, 2007), and thus have a potential of altering the ecosystems which they inhabit.

Acknowledgements

We are thankful to Rodrick Dimech and to the European Federation of Sea Anglers (EFSA) Malta section, for their collaboration in providing the specimen for research.

References

- Allen, G.R., 1991. *Damselfishes of the world*. Mergus Publishers, Melle, Germany. 271 pp.
- Allen, G.R., Woods, L.P., 1980. A review of the damselfish genus *Stegastes* from the Eastern Pacific with description of a new species. *Records of the Western Australian Museum*, 8, 171-198.
- Azzurro, E., Broglio, E., Maynou, F., Bariche, M., 2013. Citizen science detects the undetected: the case of *Abudefduf saxatilis* from the Mediterranean Sea. *Management of Biological Invasions* 4 (2), 167-170.
- Bessa, E., Dias, J.F., Souza, A.M. de, 2007. Rare data on a rocky shore fish reproductive biology: sex ratio, length of first maturation and spawning period of *Abudefduf saxatilis* (Linnaeus, 1758) with notes on *Stegastes variabilis* spawning period (Perciformes: Pomacentridae) in São Paulo, Brazil. *Brazilian Journal of Oceanography*, 55, 199-206.
- Brunt, M.A., Davies, J.E. (Eds.), 1994. *The Cayman Islands: Natural History and Biogeography*. Springer, 604 pp.
- Carpenter, K.E. (Ed.), 2002. The Living Marine Resources of the Western Central Atlantic: volume 3 - Bony fishes part 2 - (Opistognathidae to Molidae), sea turtles and marine mammals. *FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists Special Publication*, No. 5, Food and Agriculture organization of the United Nations (FAO), Rome.
- Ceccarelli, D.M., 2007. Modification of benthic communities by territorial damselfish: a multi-species comparison. *Coral Reefs*, 26, 853-866.
- Cooper, J., Smith, L.L., Westneat, M.W., 2009. Exploring the radiation of a diverse reef fish family: Phylogenetics of the damselfishes (Pomacentridae), with new classifications based on molecular analyses of all genera. *Molecular Phylogenetics and Evolution*, 52, 1-16.
- Deidun, A., Castriota, L., 2014. First record of *Abudefduf* cfr *saxatilis* Linnaeus, 1758 (Perciformes:Pomacentridae) from the Maltese Islands (Central Mediterranean). *BioInvasions Records*, 3 (1), 53-56.
- Dulčić, J., 2005. Biometric properties of damselfish, *Chromis chromis* (Osteichthyes: Pomacentridae) from the middle Adriatic. *Acta Adriatica*, 46, 91-98.
- Evans, J., Barbara, J., Schembri, P.J., 2015. Updated review of marine alien species and other 'newcomers' recorded from the Maltese Islands (Central Mediterranean). *Mediterranean Marine Science*, 16 (1), 225-244.
- Feitosa, J.L.L., Concentino, A.M., Teixeira, S.F., Ferreira, B.P., 2012. Food resource use by two territorial damselfish (Pomacentridae: *Stegastes*) on South-Western Atlantic algal-dominated reefs. *Journal of Sea Research*, 70 (1), 42-49.
- Ferreira, C.E.L., Gonçalves, J.E.A., Coutinho, R., Peret, A.C., 1998. Herbivory by the Dusky Damselfish *Stegastes fuscus* (Cuvier, 1830) in a tropical rocky shore: effects on the benthic community. *Journal of Experimental Marine Biology and Ecology*, 229 (2), 241-264.
- Froese, R., Pauly, D., 2013-2015. FishBase. URL <http://www.fishbase.us> (accessed 10.10.13, 1.05.15).
- Galil, B.S., 2006. Shipwrecked – Shipping impacts on the biota of the Mediterranean Sea. Chapter 3 in Davenport, J. and J. L. Davenport (eds.), *The Ecology of Transportation: Managing Mobility for the Environment*. Springer publishers, The Netherlands. 392 pp.
- Gobler, C.J., Thibault, D.B., Davis, T.W., Curran, P.B., Peterson, B.J. *et al.*, 2006. Algal assemblages associated with *Stegastes* sp. territories on Indo-Pacific coral reefs: Characterization of diversity and controls on growth. *Journal of Experimental Marine Biology and Ecology*, 336, 135-145.
- Golani, D., 2010. Colonization of the Mediterranean by Red Sea fishes via the Suez Canal-Lessepsian migration. In: Golani, D. and B. Appelbaum-Golani (Eds.), *Fish invasions of the Mediterranean Sea: change and renewal*. Sofia-Moscow: Pensoft Publishers. pp. 145-188.
- Golani, D., Orsi-Relini, L., Massuti, E., Quignard, J.P., 2012. *CIESM - Atlas of Exotic Fishes - List* [WWW Document].

- URL <http://www.ciesm.org/atlas/appendix1.html> (accessed 10.5.13).
- Goren, M., Galil, B.S. 1998. First record of the Indo-Pacific, coral-reef fish *Abudefduf vaigiensis* (Quoy & Gaimard, 1825) in the Levant. *Israel Journal of Zoology*, 44 (1), 57-59.
- Hinds, P.A., Ballantine, D.L., 1987. Effects of the Caribbean threespot damselfish, *Stegastes planifrons* (Cuvier), on algal lawn composition. *Aquatic Botany*, 27 (4), 299-308.
- Hutchings, P., Kingsford, M., Hoegh-Guldberg, O., (Eds.), 2008. *The Great Barrier Reef: Biology, Environment and Management*. CSIRO Publishing, Melbourne. 378 pp.
- Jribi, I., Bradai, M., 2012. First record of the Lessepsian migrant species *Lagocephalus sceleratus* (Gmelin, 1789) (Actinopterygii: Tetraodontidae) in the Central Mediterranean. *BioInvasions Records*, 1 (1), 49-52.
- Letourneur, Y., Galzin, R., Harmelin-Vivien, M., 1997. Temporal variations in the diet of the damselfish *Stegastes nigricans* (Lacepède) on a Réunion fringing reef. *Journal of Experimental Marine Biology and Ecology*, 217 (1), 1-18.
- Lipej, L., Mavri, B., Dulcic, J., 2014. First record of *Chrysiptera cyanea* (Quoy and Gaimard, 1825) (Perciformes: Pomacentridae) in the Mediterranean Sea. *Journal of Applied Ichthyology*, 30 (5), 1-3.
- Litsios, G., Pellissier, L., Forest, F., Lexer, C., Pearman, P.B. et al., 2012. Trophic specialization influences the rate of environmental niche evolution in damselfishes (Pomacentridae). *Proceedings of the Royal Society B: Biological Sciences*, 279 (1743), 3662-3669.
- Martin, A. P., Palumbi, S.R., 1993. Protein evolution in different cellular environments: cytochrome b in sharks and mammals. *Molecular Biology and Evolution*, 10 (4), 873-891.
- McEachran, J.D., Fechhelm, J.D., 2010. *Fishes of the Gulf of Mexico*, Volume 2, Scorpaeniformes to Tetraodontiformes. University of Texas Press, 1009 pp.
- Monteiro-Neto, C., De Andrade Cunha, F.E., Carvalho Nottingham, M., Araujo M.E., Lucena Rosa, E. et al., 2003. Analysis of the marine ornamental fish trade at Ceara State, northeast Brazil. *Biodiversity and Conservation* 12, 1287-1295.
- Osório, R., Rosa, I. L., Cabral, H., 2006. Territorial defence by the Brazilian damsel *Stegastes fuscus* (Teleostei: Pomacentridae). *Journal of Fish Biology*, 69 (1), 233-242.
- Palumbi, S.R., 1996. Nucleic Acids II: The Polymerase Chain Reaction, p. 205-247. In: *Molecular Systematics*. D. M. Hillis, C. Moritz, and B. K. Mable (Eds.). Sinauer Associates, Massachusetts. 655 pp.
- Papavasoulou, I., Vardakas, L., Perdikaris, C., Kommatas, D., Paschos, I., 2014. Ornamental fish in pet stores in Greece: a threat to biodiversity? *Mediterranean Marine Science*, 2014, 15 (1), 126-134: Supplementary data.
- Psomadakis, P. N., Scacco, U., Consalvo, I., Bottaro, M., Leone, F. et al., 2009. New records of the Lessepsian fish *Fistularia commersonii* (Osteichthyes: Fistulariidae) from the central Tyrrhenian Sea: signs of an incoming colonization? *JMBA* 2, *Biodiversity Records*, :e49. doi: 10.1017/S1755267209000566.
- Santangelo, N., Itzkowitz, M., Richter, M., Haley, M. P., Haley Michael, P., 2002. Resource attractiveness of the male beaugregory damselfish and his decision to court or defend. *Behavioral Ecology*, 13 (5), 676-681.
- Souissi, J. Ben, Rifi, M., Ghanem, R., Ghazzi, L., Boughedir, W. et al., 2014. *Lagocephalus sceleratus* (Gmelin, 1789) expands through the African coasts towards the Western Mediterranean Sea : a call for awareness. *Management of Biological Invasions* 5 (4), 357-362.
- Souza, A.T., Ilarri, M.I., Medeiros, P.R., Sampaio, C.L.S., Floeter, S.R., 2011. Unusual colour patterns of territorial damselfish (Pomacentridae: *Stegastes*) in the south-western Atlantic. *Marine Biodiversity Record*, 4, e101.
- Steele, J.H., Thorpe, S.A., Turekian, K.K., (Eds.), 2010. *Marine Ecological Processes: A derivative of the Encyclopedia of Ocean Sciences*. Academic Press, London. 642 pp.
- Tang, K.L., 2001. Phylogenetic relationships among damselfishes (Teleostei: Pomacentridae) as determined by mitochondrial DNA data. *Copeia*, 3, 591-601.
- Tardent P., 1959. Capture d'un *Abudefduf saxatilis vaigiensis* Q. und G. (Pisces, Pomacentridae) dans le Golfe de Naples. *Revue Suisse de Zoologie*, 66, 347-351.
- Tsadok, R., Rubin-Blum, M., Shemesh, E., Tchernov, D., 2015. On the occurrence and identification of *Abudefduf saxatilis* (Linnaeus, 1758) in the easternmost Mediterranean Sea. *Aquatic invasions*, 10 (1), 101-105.
- Vacchi, M., Chiantore, M.C., 2000. *Abudefduf vaigiensis* (Quoy & Gaimard, 1825): a tropical damselfish in Mediterranean Sea. *Biologia Marina Mediterranea*, 7 (1), 841-843.
- Vella, A., 2014a. Conservation research reports new alien species and declining local species in our sea. The *Maritime Directory Website* news: <http://www.maritimedirectory.com/mt/newsread.asp?l=e&ID=3037> issued on the 16th of January 2014. (accessed 3rd February 2014).
- Vella, A., 2014b. Conservation research reports new alien species and declining local species in our sea. *The University of Malta Website* news: <http://www.um.edu.mt/newson-campus/researchinitiatives/archive/newaliendeclininglocalspecies> (accessed 3rd February 2014).
- 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 (1462), 1847-57.
- Weigt, L.A., Baldwin, C.C., Driskell, A., Smith, D. G., Ormos, A. et al., 2012. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. *PLoS ONE*, 7 (7), e41059.