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Natural hybrid of ♀ *Diplodus cervinus* and ♂ of *D. levantinus* (Teleostei: Sparidae) from the Mediterranean coast of Israel

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Abstract

A morphomeristic and genetic analysis of an unusual specimen of *Diplodus* (Family: Sparidae) that was collected in the Mediterranean coast of Israel revealed that it is a hybrid of *Diplodus cervinus* female and *D. levantinus* male. Coloring, lip formation and teeth arrangement were intermediate between the parental species.

Keywords: Hybrid, *Diplodus cervinus, Diplodus levantinus,* Sparidae, Mediterranean.

Introduction

The family Sparidae is commercially important on the Mediterranean coast of Israel. The family consists of 23 species in this region, out of these, two (*Crenidens crenidens* and *Rhabdosargus haffara*) arrived from the Red Sea via the Suez Canal (Lessepsian migrants) (Golani *et al.,* 2006). The genus *Diplodus* consists of six species in the Levant. *Diplodus annularis* (Linnaeus, 1758), *D. cervinus* (Lowe, 1841), *D. puntazzo* (Cetti, 1777), *D. sargus* (Linnaeus, 1758) and *D. vulgaris* (Geoffroy Saint-Hilaire, 1817). Recently, Fricke *et al.* (2016) revealed that the south Israeli population of the formerly considered *Diplodus sargus* is actually a distinct species, *Diplodus levantinus.* All species of this genus in this region can be identified easily based on their external morphology and color pattern.
On 18 May 2013, a 139 mm SL unusual specimen of the genus *Diplodus* was speared at a depth of 2-3 m at the shore of Ma'agan Michael at the Mediterranean coast of Israel (32^0 33'38"N $34^0$54'27" E). The external appearance of this species was intermediate between several species of the genus. The purpose of this study was to determine, using morphological and molecular tools, the taxonomic status and identify this presumed hybrid specimen.

Materials and Methods

Sample collection

Thirteen specimens of *Diplodus* were collected from different locations and deposited in the Fish Collection of The Hebrew University (HUJ): two specimens of *D. noct* (Valenciennes, 1830) from Eilat, Red Sea (HUJ 20137) and eleven from the Mediterranean Sea: two *D. annularis* (Linnaeus, 1758) from Jaffa (HUJ 20391), one *D. vulgaris* (Geoffroy Saint-Hilaire, 1817) from Jaffa (HUJ 20281), one *D. puntazzo* (Cetti, 1777) from Michmoret (HUJ 20371), two *D. levantinus* Fricke, Golani and Appelbaum-Golani, 2016 from Jaffa (HUJ 20282), three *D. levantinus* (HUJ 20257) from Sdot-Yam, one *D. cervinus* (Lowe, 1841) from Akko (HUJ 19723) and one specimen of *Diplodus* sp., suspected to be a hybrid from Ma'agan Michael (HUJ 20233). One specimen of *Priacanthus sagittarius* Starnes, 1988 from Jaffa, (HUJ 20015) was collected as well.
Biometrical counts and measurements follow Hubbs & Lagler (1947).

Molecular analysis

Adult fish muscles (about 50 mg) were used for DNA sample preparation using the Accu-Prep® genomic DNA extraction kit (Bioneer, Daejeon, Korea).
PCR and sequencing.
Approximately 650 bp were amplified from the 5′ region of the mitochondrial cytochrome c oxidase subunit I gene (COI) using the following primers (Ward *et al*., 2005):
FishF1: 5′ TCAACCAACCACAAAGACATTGGCAC 3′
FishR1: 5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′
PCR reactions were carried out in 25 µl reaction volumes containing 1× PCR buffer (including 1.5 mM MgCl2), 0.2 mM of each dNTP, 1 µM of each primer, 1 unit of Super-Term Taq polymerase (Hoffmann-La Roche), and about 100 ng of template DNA. PCR reactions were processed in a Bio-Rad C-1000 thermal cycler with the following thermal regime: an initial step of 2 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 57°C and 1 min at 72°C, followed by 3 min at 72°C and then held at 15°C. PCR products were visualized on 1.5% agarose gels and sequenced bidirectionally using the PCR

primers on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Approximately 500 bp were amplified from the nuclear region between the 18S and 28S ribosomal RNA genes (containing 5.8S ribosomal RNA gene, and internal transcribed spacer 2) using the following self-designed primers that are reported here for the first time:
18S-1762-F: 5′ AAGTCGTAACAAGGTTTCCGT 3′
28S-59-R: 5′ TCCTCCGCTTAGTAATATGCTTAAA 3′
PCR reactions were carried out as described above with the following thermal regime: an initial step of 2 min at 95°C followed by 32 cycles of 45 s at 94°C, 45 s at 55°C and 45 s at 72°C, followed by 3 min at 72°C and then held at 15°C. Some of the reactions (for *D. cervinus*) needed to be amplified with a 50^0C annealing temperature instead of 55^0C. PCR products were visualized and sequenced as described above.
Data analysis - Sequences were aligned using CLUSTAL W (Thompson *et al*., 1994) in BioEdit ver. 7.0.9.0 (Hall, 1999). Neighbor-joining analysis was carried out using PHYLIP version 3.69 (Felsenstein, 2009). Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016). Trees were built using the Neighbor-Joining method (Saitou & Nei, 1987). Bootstrap test (500 replicates) was used to calculate the percentage of replicate trees in which the associated taxa clustered together (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004).

Results and Discussion

Description of the Diplodus *hybrid (HUJ 20233)*

Body ovate, moderately deep (2.04 times in SL) and slightly compressed**.** Head 3.19 times in SL. Dorsal profile of head straight. Pointed snout (2.38), eye diameter (3.63), interorbital (2.66), all times in SL. Mouth slightly oblique, maxilla reaching back almost to the vertical of eye. Lips relatively developed. A row of nine incisor teeth in the anterior of upper jaw and ten in the lower jaw. Nine small molariform teeth arranged in two rows in the upper jaw. In the lower jaw, there are 14 molars on the left side and 12 on the right side. In both sides the teeth in the inner row are larger and there are two minute teeth on the exterior side. Continuous dorsal fin with 11 spines and 12 soft rays. Anal fin with three spines and 11 soft rays. Caudal fin forked. Pectoral fin long and pointed with 17 rays, reaching back beyond anus. Pelvic fin with a single spine and five rays, its origin slightly beyond pectoral fin origin.
Color: Body silvery with six vertical black bars not reaching the ventral surface. A black streak on the anterior part of the caudal peduncle. A dark blotch under the eye and dark posterior edge of the operculum. Dorsal and caudal fins grey, anal and pelvic fins dark grey. Pectoral fin slightly orangeish.
All COI sequences obtained from our samples (GenBank # MF464085-098) matched sequences of the respective species previously reported in GenBank, except for the sequence of *D. levantinus* that has not been described before (BLAST-NCBI was used as our search engine). The genetic distances between the different samples are presented in Table 1. The two markers show the same differences between species though the COI is more sensitive and gives higher values. The difference between *D. levantinus* and *D. cervinus* are very clear: 6.7% using the COI and 2.1% using the ITS2. As expected, the mtDNA phylogenetic tree (Fig. 1) clearly demonstrates the differences between species of the genus *Diplodus*. The COI

Table 1. Genetic differences between this study's samples using COI (A) or ITS2 (B) as markers. Genetic distances were calculated using DNADIST version 3.5c of BioEdit ver. 7.0.9.0 (Hall, 1999).

A. COI

	P. sagittarius	*D. annularis*	*D. vulgaris*	*D. noct*	*D. puntazzo*	*D. Hybrid*	*D. levantinus*
D. annularis*	0.235						
D. vulgaris	0.245	0.109					
D. noct*	0.236	0.112	0.083				
D. puntazzo	0.253	0.114	0.072	0.065			
D. Hybrid	0.252	0.127	0.092	0.062	0.069		
D. levantinus**	0.227	0.110	0.082	0.008	0.062	0.067	
D. cervinus	0.252	0.127	0.092	0.062	0.069	0	0.0668

* 2 Specimen
** 5 specimen

B. ITS2

	P. sagittarius	*D. annularis*	*D. vulgaris*	*D. noct*	*D. puntazzo*	*D. Hybrid*	*D. levantinus*
D. annularis*	0.462						
D. vulgaris	0.413	0.137					
D. noct*	0.399	0.133	0.019				
D. puntazzo	0.423	0.142	0.045	0.032			
D. Hybrid	0.399	0.133	0.019	0	0.032		
D. levantinus**	0.399	0.133	0.019	0	0.032	0	
D. cervinus	0.426	0.142	0.029	0.021	0.029	0.021	0.021

* 2 Specimen
** 5 specimen

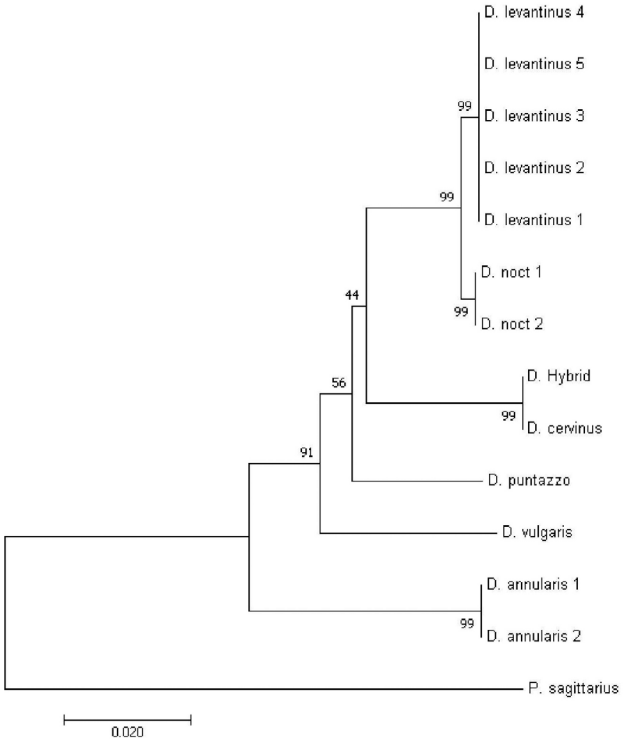

Fig. 1: Evolutionary relationships of *Diplodus* spp. from the Mediterranean coast of Israel, based on the mitochondrial gene COI (Cytochrome c oxidase I). The optimal tree with the sum of branch length = 0.26755226 is shown. Bootstrap test percentages are shown next to the branches.

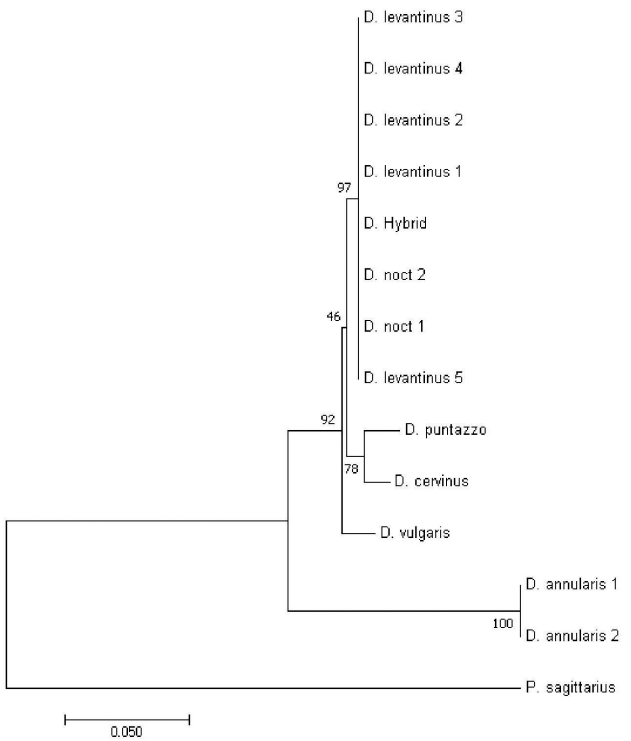

Fig. 2: Evolutionary relationships of *Diplodus* spp. from the Mediterranean coast of Israel, based on the nuclear region ITS2 (internal transcribed spacer 2). The optimal tree with the sum of branch length = 0.48756071 is shown. Bootstrap test percentages are shown next to the branches.

sequence of the suspected hybrid had 100% similarity to *D. cervinus* and since the COI is maternal inheritance, this implies that the mother of the hybrid was *D. cervinus*. In order to try and find the paternal source of the hybrid we looked for a nuclear marker. Since no previous data was available, we designed primers and got sequences for the region between the 5.8S and the 28S ribosomal RNA genes. These primers were not general enough and DNA from some of the species, like *D. cervinus*, were not amplified, using the initial PCR protocol. After reducing the annealing temperature to 50^0C we managed to get the *D. cervinus* to amplify as well. All ITS2 sequences obtained from our samples (GenBank # MF464099-112) did not match any sequences in GenBank. The phylogenetic tree that we have constructed demonstrates the differences between species of the genus *Diplodus* (Fig. 2). A sequence that was amplified from the suspected hybrid's DNA had 100% similarity to *D. levantinus*. Since we already knew that the hybrid inherited its mtDNA from a *D. cervinus* mother, we have concluded that the nuclear ITS2 sequence was inherited from a *D. levantinus* father. We have therefore concluded that the paternal parent of the hybrid might be a *D. levantinus* male. Bias caused by differential template annealing in the amplification of target mixtures by PCR is well documented, especially for 16S amplification in environmental samples (Suzuki & Giovannoni, 1996). We saw no need in proving again that the other parent was *D. cervinus*.

Hybridization among fishes is a common phenomenon and well-documented in literature. Most cases of known hybridizations were reported from freshwater fishes (Schwartz, 1981). The reason for this is that, in most cases, the speciation of freshwater is much more recent and presumably the reproductive barriers between species were not completed, enabling hybridization. However, among marine fish, hybridization is not unknown. Many cases have been reported (Srinivasa Rao & Lakshmi, 1999; Roberts *et al.,* 2011). Most hybridization of marine fish is reported from families of colorful species such as Butterflyfishes (Chaetodontidae), Angelfishes (Pomacanthidae) and Wrasses (Labridae) (Randall & Miroz, 2001). These authors, Pyle & Randall (1994) and Randall & Frische (2000) postulated that the rate of hybridization is not necessarily higher than that of less colorful families where detection of hybrids is more difficult. Hybridization between two species of Sparidae were recorded in the Mediterranean by Jug-Dujakovich & Glamuzina (1993) and Kraljević & Dulčić (1999) from Croatia.

The specimen under study has intermediate characteristics between *Diplodus cervinus* and *D. levantinus*. It had six distinct bars, the space between them being wider than the bars' width, while *D. cervinus* has only four wide bars with narrow spaces between them; *D. levantinus* has eight narrow bars (Golani *et al.,* 2006; Fricke *et al.,* 2016). In addition, the hybrid has slightly developed lips, while *D. cervinus* has distinctly thick lips and *D. levantinus* has un-

distinguishable lips (Fig. 3). Also, the teeth arrangement of the hybrid is between the two parental species. *D. cervinus* has a row of ten incisor teeth in the upper jaw, *D. levantinus* has eight incisor teeth, while the hybrid has nine, the molar teeth being in an intermediate position. (Fig. 4).
In most cases of natural hybridization, specimens are aberrant or sterile. However, in some cases the hybrids are as successful as their parental species (Lotan & Ben-Tuvia, 1996). In these cases, the population of the successful hybrids could become part of the speciation process leading to the development of a new species (Pennisi, 2016).

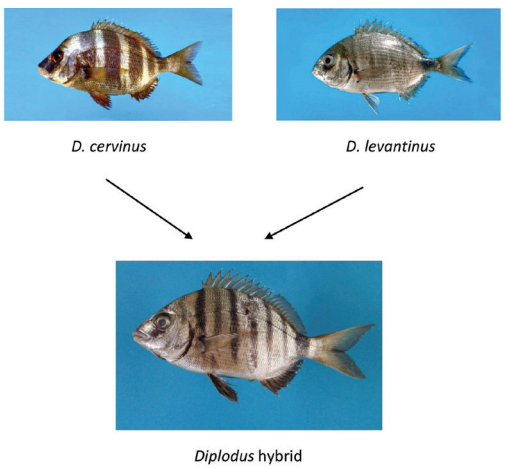

Fig. 3: The two parental species (*Diplodus cervinus* and *D.* levantinus) and their hybrid.

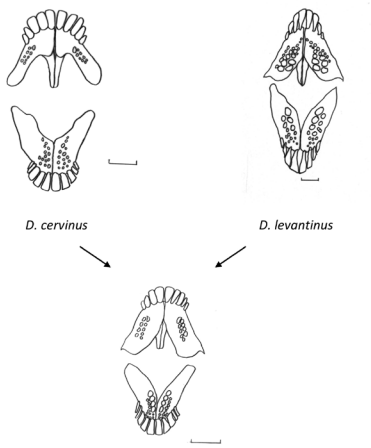

Fig. 4: Dentition of the two parental species (*Diplodus cervinus* and *D. levantinus*) and their hybrid. Scales indicate 3 mm.

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References

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783-791.

Felsenstein, J., 2009. *PHYLIP© - Phylogeny Inference Package (version 3.69)*. Department of Genome Sciences, University of Washington, Seattle. Computer program.

Fricke, R., Golani, D., Appelbaum-Golani, B., 2016. *Diplodus levantinus* (Teleostei: Sparidae), a new species of sea bream from the southeastern Mediterranean Sea of Israel, with a checklist and a key to the species of the *Diplodus sargus* species group. *Scienta Marina*, 80(3), 305-320.

Golani, D., Öztürk, B., Başusta, N., 2006. *Fishes of the Eastern Mediterranean*. Turkish Marine Research Foundation Publication no. 24, Istanbul, Turkey. 259 pp.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hubbs, C.L., Lagler, K.F., 1947. *Fishes of the Great Lakes Region*. Bulletin Cranbrook Institute of Science. Bloomfield Hills, Michigan. 26: VI+186 pp.

Jug-Dujakovich, J., Glamuzina, B., 1993. Intergeneric hybridization in Sparidae. *Journal of Applied Aquaculture,* 2 (1), 105-114.

Kraljević, M., Dulčić, J., 1999**.** Intergeneric hybridization in Sparidae (Pisces: Teleostei): *Dentex* (*Dentex*) *dentex* female x *Pagrus major* male and *P. major* female x *D. dentex* male. *Journal of Applied Ichthyology*, 15, 171-175.

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870-1874.

Lotan, R., Ben-Tuvia A., 1996. Distribution and reproduction of Killifish *Aphanius dispar* and *A. fasciatus* and their hybrids in the Bardawil Lagoon on the Mediterranean coast of Sinai, Egypt. *Israel Journal of Zoology,* 42, 203-213.

Pennisi, E., 2016. Shaking up the tree of life. *Science*, 354, 817-821.

Pyle, R.L., Randall, J.E., 1994. A review of hybridization in marine angelfishes (Perciformes: Pomacanthidae). *Environmental Biology of Fishes,* 41, 127-145.

Randall, J.E., Frische, J.. 2000. Hybrid surgeonfishes of the *Acanthurus achilles* complex. *Aqua, Journal of Ichthyology and Aquatic Biology*, 4 (2), 51-56.

Randall, J.E., Miroz, A., 2001. *Thalassoma lunare* X *Thalassoma rueppelli*, hybrid labrid fish from the Red Sea. *Aqua, Journal of Ichthyology and Aquatic Biology*, 4 (4), 131-134.

Roberts, D.G., Gray, C.A., West, R.J., Ayre, D.J., 2011. Temporal stability of hybrid swarm between the migratory marine and estuaries fishes *Acanthopagrus australis* and *A. butcheri*. *Marine Ecology Progress Series*, 421, 199-204.

Saitou, N., Nei, M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.

Schwartz, F.T., 1981. *World literature of fish hybrids with an analysis by family, species and hybrid. Suppl 1*. NOAA Technical Report NMFS SSRF-750. 507 pp.

Srinivasa Rao, K., Lakshmi, K., 1999. Cryptic hybridization in marine fishes: significance of narrow hybrid zone identifying stable hybrid populations. *Journal of Natural History*, 33 (8), 1237-1259.

Suzuki, M.T., Giovannoni, S.J., 1996. Bias caused by template

annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Applied and Environmental Microbiology*, 62 (2), 625-630.

Tamura, K., Nei M., Kumar S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)*, 101, 11030-11035.

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 (22), 4673-4680.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D., 2005. DNA barcoding Australia's fish species. *Philosophical Transaction of the Royal Society of London B,* 360, 1847-1857.