PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia Biologica (Kraków), vol. 63 (2015), No 1
doi: 10.3409/fb63_1.19

Genetic Identification of a Rare Record of *Ommastrephes Bartramii* (Cephalopoda: Ommastrephidae) from the Eastern Adriatic Sea*

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Accepted December 15, 2014

FRANJEVIĆ D., SKARAMUCA D., KATAVIĆ V., RAJEVIĆ N., SKARAMUCA B. 2015. Genetic Identification Of a rare record of *Ommastrephes Bartramii* (Cephalopoda: Ommastrephidae) from the eastern Adriatic Sea. Folia Biologica (Kraków) **63**: 19-23.

The neon flying squid *Ommastrephes bartramii* Lesueur, 1821 belongs to the Ommastrephidae, Cephalopoda family. The family Ommastrephidae (Mollusca: Cephalopoda) includes many commercially important species, dispersed around the world. The Ommastrephidae family is conventionally divided into three subfamilies (Illicinae, Todarodinae, and Ommastrephinae). We report a specimen of neon flying squid caught in the winter 2013 at Luka Šipanska, Island of Šipan, Croatia and identified at the genetic level using the standard mitochondrial COI barcode region. This record represents the first genetic identification of a neon flying squid from the Adriatic Sea.

Key words: COI barcoding, mitochondrial DNA, eastern Adriatic Sea, *Ommastrephes bartramii*, rare record.

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According to GAMULIN-BRIDA and ILIJANIĆ (1965) as well as BELLO (1990), the distribution of *Ommastrephes bartramii* is cosmopolitan; however it is exceptionally rare in the Adriatic. This communication represents the first genetic identification ever of this species from the eastern Adriatic Sea.

Ommastrephes bartramii, commonly known as the neon flying squid, akaika, red squid, red ocean squid, red flying squid, flying squid, and Bartram's squid (among many other names), is the most broadly distributed species in the Ommastrephidae family with a circumglobal subtropical to temperate distribution (MURATA 1990). The Ommastrephidae are characterized by a muscular body, an inverted T-shaped funnel locking cartilage, biserial suckers on the arms and tetraserial suckers on the tentacular clubs, with the exception of the genus Illex that has eight rows of dactylus suckers. The family comprises three subfamilies (Illicinae, Todarodinae and Ommastrephinae) according to the structure of the funnel groove (ROPER *et al.* 1984), and at present a total of 23 species are recognized (OKUTANI 2005).

It is found circumglobally in subtropical, temperate waters and sustains important fisheries in the North Pacific, but it is rarely encountered in the Mediterranean Sea. This species is a powerful,

*Supported by the Adris Foundation project (DF), Croatian Ministry of Science Education and Sports projects 108-1080229-0341 (VK) and 275-0010501-0856 (BS).

epipelagic, jet-propelled swimmer equipped with a strongly muscular mantle for maximum thrust and short, muscular, triangular fins for stabilization and maneuvering. *Ommastrephes bartramii* is a major predator which feeds on fishes in the transitional waters of the central north Pacific Ocean. *Ommastrephes bartramii* occurs at sea surface temperatures from 10°C to 25°C, generally over bottom depths greater than 200 m.

The population of *O. bartramii* in the North Pacific annually makes a round-trip migration between its subtropical spawning grounds and its northern feeding grounds near the subarctic boundary (BOWER & ICHII 2005). The population consists of two seasonal cohorts, an autumn spawning cohort and a winter-spring spawning cohort. All past findings of this species in the Adriatic Sea and its family have been based solely on morphological determination.

DNA sequences have become a major source of new information for advancing our understanding of evolutionary and genetic relationships. DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify if it belongs to a particular species. http://en.wikipedia.org/wiki/DNA_barcoding - cite_note-Hebert2003-1 DNA barcoding provides an efficient method for species-level identifications and contributes powerfully to taxonomic and biodiversity research (HEBERT *et al.* 2003). The Folmer region (FOLMER *et al.* 1994), of the mitochondrial cytochrome c oxidase subunit I (COI) gene was proposed as a universal DNA barcode region for animals.

Material and Methods

A female specimen of the *Ommastrephes bartramii* was caught at 1.5 m depth with a spear on February 25, 2013 at Luka Šipanska, Island of Šipan, Croatia (42°43'25"N 17°52'33"E) (Fig. 1). The specimen had a body weight of 9 kg and was 1.3 m in length without tentacles (Fig. 2).

DNA was extracted from a tentacular sucker using a standard phenol/chlorophorm protocol used for DNA extraction from teeth (PFEIFFER *et al.* 1998) Lysis buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 50 mM EDTA, pH 8.0, 0.5% SDS, pH 8.0, and 20 μl proteinase K at 20 mg/ml) was added to the tissue sample, vortexed and incubated at 56°C

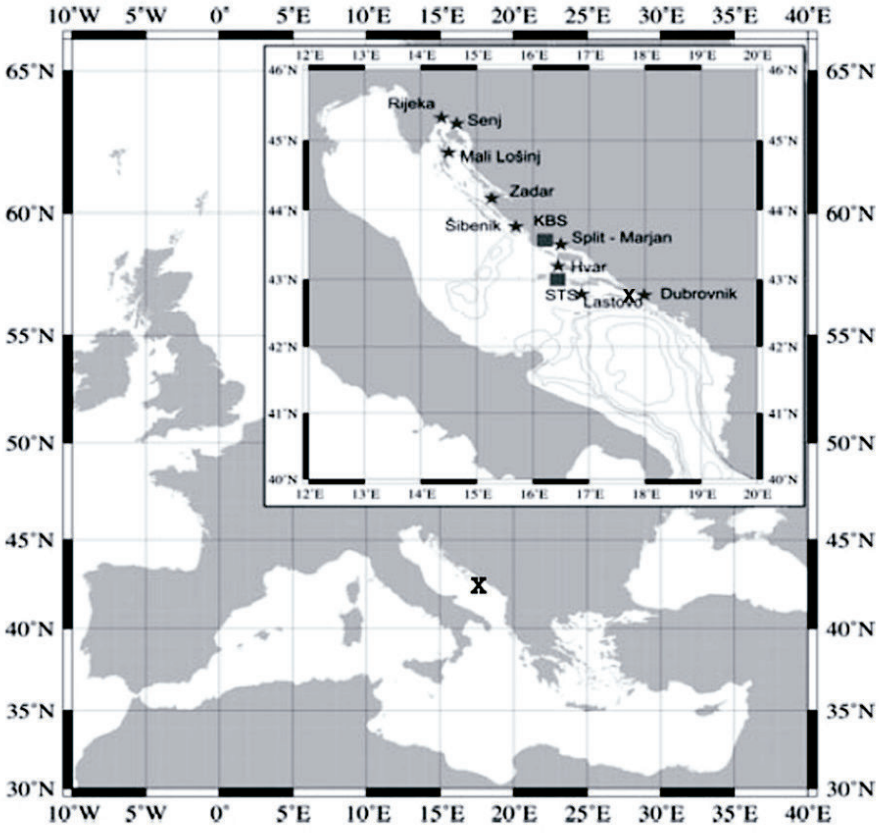

Fig. 1. General map of the Mediterranean with a magnified area of the Adriatic Sea. Mark X shows the spot where the sample of neon flying squid was caught.

Fig. 2. Specimen of *Ommastrephes bartramii* caught at Luka Šipanska, Island of Šipan, Croatia.

overnight. This was followed by adding the extraction buffer (phenol:chlorophorm:isopropranol at a 25:24:1 ratio) to the sample. The sample was then vortexed and centrifuged for 15 seconds at 12000 g. The aqueous layer was transferred to a sterile tube and DNA was precipitated by ethanol. The resulting DNA pellet was dried and then dissolved in water. All chemicals used in DNA extraction, isolation, and purification were supplied by Sigma-Aldrich (Taufkirchen, Germany). The PCR product was amplified via a Mastercycler Personal (Eppendorf, Germany) using Hot-MasterMix (Eppendorf, Germany) in 50 μl reactions containing: 25 μl HotMasterMix-a, 22 μl mQ H2O, 1 μl of DNA and 1 μl of each primer. The COI gene fragment was amplified using primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACC AAAAAATCA-3') from FOLMER *et al.* (1994). PCR was performed using the following cycling settings: 5 min predenaturation at 95°C followed by 1 min at 94°C, 1 min at 50 °C and 1 min at 72°C, for 35 cycles followed by 72°C final elongation for 10 min. PCR products were purified with the Qiagen PCR Quick Purification KIT (Qiagen, Germany). For sequencing purposes we used services of Macrogen Europe (Amsterdam, The Netherlands). The sequence chromatogram was viewed and edited manually using Chromas Lite 2.0 (Technelysium Pty., South Brisbane, Australia). Forward and reverse sequences were checked for base ambiguity in BioEdit 7.2 (HALL 1999). The length of the amplified COI fragment was 666 bp long.

The mitochondrial COI sequence of neon flying squid caught near the Island of Šipan, Croatia has GenBank accession number KF212462.

Results and Discussion

Results retrieved using the BLAST service showed a high sequence similarity to nine sequences of neon flying squid from GenBank. The intraspecific maximum identity score was 94% corroborating that the sample was a neon flying squid from the Adriatic Sea while interspecific identity score with some species of the same family ranged from 84% to 82% namely: *Sthenoteuthis oualaniensis* 84%, *Hyaloteuthis pelagica* 83%, *Illex argentinus* 83% and *Nototodarus hawaiiensis* 82%.

The distribution and abundance of *Ommastrephes bartramii* correlates with the water temperature regarding spawning (21-25°C), feeding grounds and migration routes (6-15°C) in the range of 200 m depth (BOWER & ICHII 2005). MANDIĆ (1984) found that the temperature is a very important factor in determining the distribution and dislodgment of South Adriatic cephalo-

pods. The more frequent observations of moribund spawning females at the periphery of the Cretan Sea are indicative of a spawning ground in this area. The suspected recent increase of *O. bartramii* abundance in both the northeastern and northwestern basins of the Mediterranean Sea might be due to the warming of upper sea layers which has been observed since the mid-1980s and is considered to be the main factor driving the northward expansion of warm-water species in the Mediterranean Sea (LEFKADITOU *et al.* 2011). The surface water temperatures generally range from 24°C in summer to 12°C in winter, significantly moderating the Adriatic Basin's climate. A report on Adriatic hydrology after ZORE-ARMANDA (1981) states that the general conditions of the Adriatic are quite different from those of the Ionian, a neighboring Sea. During the cold part of the year its waters are colder and less salty because of discharge in its northern part from the comparatively large rivers Po and Adige; both surface temperature and salinity increase southward. The general circulation, which is governed by the cyclonic gyre that follows a counter clock-wise pattern (BULJAN & ZORE-ARMANDA 1976; BELLO 1990), favors water exchange with the Mediterranean through the Strait of Otranto. The north-western flow prevails on the surface and intermediate layers down to about 400 m in the South Adriatic, while the south-western flow prevails in the bottom layers. It is known that incidental periods of intensive impact of the Mediterranean upon the Adriatic, called the Adriatic ingressions (ZORE-ARMANDA 1981) may be linked with some rare events in marine life such as this rare appearance of neon flying squid. These species, likely coming from the Mediterranean, enter the Adriatic following the northwestward current on their way from the Otranto Strait toward the northern Adriatic. Furthermore, it is possible that these individuals are transported from the open waters of the SE Mediterranean due to the strong currents from the south-east prevailing in these waters. Precisely, the specimen in question was caught after a storm in the shallow waters in the port of Šipanska Luka. Prevailing southeastern winds during winter and spring might transport associated animals towards the coast and would explain the appearance of this species. Our finding corresponds with increasingly frequent records, in recent years, of thermophilic fish species in Adriatic waters (DULČIĆ & GRBEC 2000; DULČIĆ *et al.* 2004). Our data may support the current interest in climate change correlated with species distributions and increase of sea temperature. The natural habitat of *O. bartramii* is below 200 m of depth and in a temperature span between 12°C and 26°C, suggesting that our specimen may have been a moribund spawning female which often show irregular distribution (LEFKADITOU *et al.* 2011).

Acknowledgements

The authors are indebted to Mr. Stanley STJEPOVIC and the fishermen for providing the sample, and Ms Sanja IVČEVIĆ for her technical expertise and DNA isolation.

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