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A Mediterranean Monk Seal Pup on the Apulian Coast (Southern Italy): Sign of an Ongoing Recolonisation?

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Abstract: The Mediterranean monk seal (*Monachus monachus*) is one of the most endangered marine mammals in the world. This species has been threatened since ancient times by human activities and currently amounts to approximately 700 individuals distributed in the Eastern Mediterranean Sea (Aegean and Ionian Sea) and Eastern Atlantic Ocean (Cabo Blanco and Madeira). In other areas, where the species is considered "probably extinct", an increase in sporadic sightings has been recorded during recent years. Sightings and accidental catches of Mediterranean monk seals have become more frequent in the Adriatic Sea, mainly in Croatia but also along the coasts of Montenegro, Albania and Southern Italy. A Mediterranean monk seal pup was recovered on 27 January 2020 on the beach of Torre San Gennaro in Torchiarolo (Brindisi, Apulia, Italy). DNA was extracted from a tissue sample and the hypervariable region I (HVR1) of the mitochondrial DNA control region was amplified and sequenced. The alignment performed with seven previous published haplotypes showed that the individual belongs to the haplotype MM03, common in monk seals inhabiting the Greek islands of the Ionian Sea. This result indicates the Ionian Islands as the most probable geographical origin of the pup, highlighting the need to intensify research and conservation activities on this species even in areas where it seemed to be extinct.

Keywords: *Monachus monachus*; Mediterranean monk seal; mitochondrial DNA; Adriatic Sea; endangered species

1. Introduction

The Mediterranean monk seal (*Monachus monachus*, Hermann 1779) is the only living representative of the genus *Monachus* [1] and one of the most endangered mammals in the world [2]. It is a medium-sized phocid that usually inhabits waters up to 200 m in depth and is closely linked to coastal habitats for reproduction [3]. After mating in water, the females make their way to land and give birth to pups in coastal caves probably as an adaptation to predation, including hunting by humans [4,5]. Parturition usually occurs during the autumn season [6–8] but, in the Cabo Blanco colony, the birth

of pups has also been observed in other months of the year as a result of favorable environmental conditions and the availability of food [6,9].

The Mediterranean monk seal was historically widespread in the Mediterranean Sea, Black Sea and Eastern Atlantic Ocean [3] but, at present the species consists of no more than 700 individuals inhabiting Ionian and Aegean waters within the Eastern Mediterranean area, the Madeira archipelago, and Cabo Blanco waters in the Eastern Atlantic [2]. The size of populations and the geographical distribution of the species have over time been impacted by several threats such as habitat deterioration, intentional or accidental killing and unusual mass mortality events [3]. Since prehistoric times *M. monachus* has been hunted by man [10,11], although intense exploitation probably occurred in the Roman and Medieval periods when monk seals were extensively hunted for their skin, oil, and meat [4,5]. Fishing is still the main cause of death of Mediterranean monk seals as many of them, considered responsible for a negative impact on fishing activities, are intentionally killed by fishermen [12] or accidentally become entangled in fishing nets (bycatch) [13]. In addition, coastal habitat degradation and increased tourism have reduced the mainland areas available for resting and pupping, putting at risk the reproductive capacity of the species [3]. In 1997, a mass mortality event was documented in Cabo Blanco highlighting the fact that, together with other stochastic events [3], viruses and toxic algal blooms [14,15] could also be important additional threats to monk seal survival.

In order to assess the conservation status of *M. monachus*, several studies have analyzed the genetic diversity levels of extant populations using nuclear and mitochondrial DNA sequences [16–19]. Both markers have highlighted very low levels of genetic diversity in Mediterranean monk seal populations, probably as a direct consequence of bottleneck events that occurred in the past [16–19]. In addition, the analysis of the most variable part of the mitochondrial DNA (mtDNA) control region has allowed the identification of three distinct sub-populations (Aegean Sea, Ionian Sea and Eastern Atlantic Ocean) characterised by different haplotypes [18]. Subsequently, the analysis of the same mtDNA marker, in both historical and current monk seal specimens, has allowed a re-evaluation of the previous result [19]. Gaubert et al. [19] showed that some mitochondrial haplotypes had a wider geographical distribution in the past and that, in agreement with previous results obtained by nuclear markers [17], there was a gene flow between populations which today seem to be genetically separate [19].

In recent years, a recovery in monk seal populations has been observed and the species has been reclassified as "Endangered" by the International Units for the Conservation of Nature (IUCN) [2]. Probably following the recovery of *M. monachus* populations, an increase in sightings has been observed in areas where the species seemed to be extinct, including North African countries, the coasts of the Levantine Sea, and those of the Adriatic-Ionian region [20]. Sightings of monk seals within the Adriatic basin are now numerous in Croatia, although vagrant individuals are also recorded in Montenegro, Albania and Apulia (Southern Italy) [20]. The presence of the Mediterranean monk seal along the Apulian coasts is not new; bone remains excavated from the Grotta Romanelli indicate that this area was a habitat for *M. monachus* individuals dating back to the Late Upper Paleolithic [21]. Over the centuries the species has progressively disappeared from Apulia, although some sightings were recorded in the Tremiti Island archipelago and in Salento in the second half of the 1900s [22] and between 2000 and the present day [20,23], indicating a probable recolonisation of this area.

On 27 January 2020, a Mediterranean monk seal pup was recovered stranded on the beach of Torre San Gennaro in Torchiarolo (Brindisi, Apulia, Italy). The young seal was debilitated and died the following day despite rescue efforts. A tissue sample was obtained, and a genetic analysis was performed in order to identify the most likely geographical origin of this monk seal individual.

2. Materials and Methods

The Mediterranean monk seal analyzed in this study (Figure 1) was recovered on the beach of Torre San Gennaro in Torchiarolo (Brindisi, Apulia, Italy) (Figure 2). After the death of the monk seal, standard measurements were taken and a necropsy was carried out by the "Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata" (IZSPB, Foggia, Italy) in order to reveal the possible cause of death for the individual. During this procedure a piece of muscle tissue was sampled, stored in absolute ethanol and sent to the Laboratory of Evolutionary Biology (Università Politecnica delle Marche, Ancona, Italy) for genetic analysis.

Figure 1. A picture of the Mediterranean monk seal pup taken before the necropsy.

Figure 2. Map of the Adriatic-Ionian region showing the haplotype distribution of the Mediterranean monk seals previously analyzed. Only data with correct geographic locations are shown. The circle indicates data from Karamanlidis et al. [18], the triangle indicates data from Gaubert et al. [19], and the star indicates the location where our sample was found.

Genomic DNA was extracted from a small slice of muscle tissue using an automated nucleic acids extractor, MagCore® HF16 (RBC Bioscience Corp., Taipei, Taiwan), with the MagCore® Genomic DNA Tissue Kit (cartridge code 401) (RBC Bioscience Corp., Taipei, Taiwan) and following manufacturer's instructions. After the DNA extraction, the hypervariable region I (HVR1) of the mtDNA control region was amplified using primer pairs designed by Karamanlidis et al. [18].

Polymerase Chain Reaction (PCR) was performed in 25 µL volume containing: 5 µL of PrimeSTAR® GXL Polymerase Buffer (5×) (Takara, Shiga, Japan), 2 µL of dNTP mix (10 mM), 2 µL of Forward and Reverse Primers mix (5 µM), 0.8 µL of PrimeSTAR® GXL DNA Polymerase (1.25 U/µL) (Takara, Shiga, Japan), 3 µL of DNA template (40 ng/µL) and 12.2 µL of ultrapure sterile water. The amplification

was carried out in a BioRad T100™ Thermal Cycler (BioRad, Hercules, CA, USA), using the following conditions: an initial denaturation step of 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C (denaturation), 30 s at 57 °C (annealing), 60 s at 72 °C (extension), and a final extension step of 7 min at 72 °C. The PCR product was run on a 2% agarose gel stained with GelRed™ (Biotium Inc., Hayward, CA, USA) to check the effectiveness of the amplification reaction and then sent to BMR Genomics (Padua, Italy) for Sanger sequencing. There, it was purified using exoSAP-IT™ (USB Corp., Cleveland, OH, USA) and sequenced in both directions on an ABIPRISM 3730XL automated sequencer (Applied Biosystems, Tokyo, Japan).

The sequence obtained was aligned on CLUSTALW [24] with those of the seven *M. monachus* haplotypes identified within the whole species range (GenBank Accession numbers in Table 1) [18,19]. The alignment was checked on BioEdit [25] and then a median-joining network (with $\varepsilon = 0$) [26] was designed using Network 10 software (Fluxus Technology Ltd., Colchester, UK, www.fluxus-engineering.com) in order to visualize the relationships between all haplotypes. In addition, with the aim of highlighting the geographical origin of the monk seal pup, information about the frequency and the geographical provenance of all haplotypes was obtained from previous studies [18,19] and added during the network construction. The information regarding the geographical origin of the haplotypes previously identified in the Adriatic-Ionian region [18,19] has also been included in a map.

Table 1. Diagnostic sites obtained from the alignment of the seven Mediterranean monk seal haplotypes. The haplotype belonging to the individual analyzed is highlighted in grey. Haplotype MM01 was used as a reference sequence. All identical nucleotides in other sequences are indicated as full stops.

		Diagnostic Sites					
Haplotype ID	**GenBank Accession No.**	**105**	**114**	**118**	**248**	**279**	**508**
MM01	KT935311	A	A	A	A	C	G
MM02	KT935307	G	.	.	.	.	.
MM03	KT935310	.	.	.	.	.	A
MMBR	MT524708	.	.	.	.	.	A
MM05	KT935309	.	.	G	.	.	.
MM06	MG570470	.	.	G	.	T	.
MM04	KT935308	.	.	G	.	.	A
MM07	MG570469	.	G	G	G	.	A

Capital letters indicate nucleotides: A—adenine, C—cytosine, G—guanine, T—thymine.

3. Results

The Mediterranean monk seal was a female weighing 22.5 kg and 118 cm long. Pelage color, weight and standard length suggest that it was a pup of about 2–4 months of age. Genetic analysis of the individual allowed us to successfully amplify and sequence a 524 bp fragment of the HVR1 of the mtDNA control region. The sequence obtained was stored in the GenBank repository with the Accession number MT524708. After the alignment of this sequence with those of the seven *M. monachus* haplotypes known so far, six diagnostic sites were identified, allowing the assignment of the pup sequence to the haplotype MM03 (Table 1). The map and the haplotype network, which include information about geographical origin of the known haplotypes, showed that the haplotype MM03 has been previously observed only in Greek populations inhabiting the Ionian Sea and in a sample from the Adriatic Sea (Figures 2 and 3).

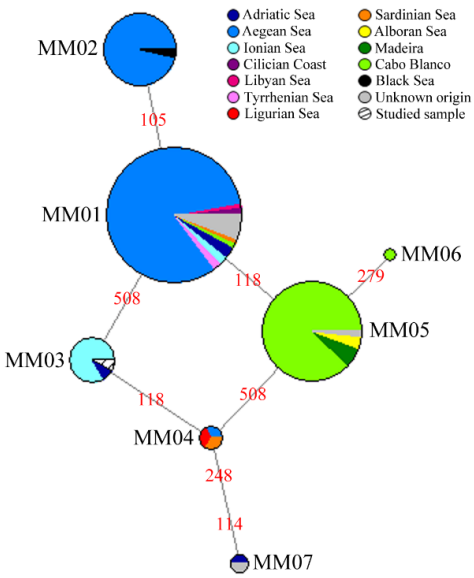

Figure 3. Median-joining network of the seven Mediterranean monk seal haplotypes. Colors indicate geographic sampling locations. Size of each circle is proportional to the haplotype frequency. Numbers in red indicate mutated positions.

4. Discussion

A Mediterranean monk seal found along the Apulian coast (Southern Italy) at the end of January 2020 was genetically analyzed in this study. The amplification and sequencing of the HVR1 fragment of the mtDNA control region have allowed the haplotype identification of the monk seal. The individual studied belonged to the haplotype MM03, one of the least common within the current distribution range of the species [18,19]. The haplotype MM03 was previously found only in 10 individuals sampled from the Greek islands (Kefalonia and Zakynthos) in the Ionian Sea [18] and from one individual in the Adriatic Sea [19]. The latter was a museum specimen which had been caught along the west coast of Brac (Croatia) in 1914 [19]. Because of the absence of reproductive populations of the species, the Mediterranean monk seal is currently considered "probably extinct" within the Adriatic Sea and the few individuals recorded in this basin are usually considered as "vagrants", individuals coming from areas where the species is more abundant and reproductively active [20,23]. While the Adriatic waters are only sporadically inhabited by Mediterranean monk seals, the Ionian Islands still host several *M. monachus* individuals. In fact, one of the largest populations of this species is found in the Eastern Mediterranean Sea and approximately 300–400 individuals are distributed along the Greek coasts [3]. The Ionian Islands are important sites for the conservation of the Mediterranean monk seal because they are characterised by a heterogeneous coastal habitat, which includes both open sandy beaches and several rocky areas, with marine caves suitable as resting and breeding grounds for monk seals [27,28]. Studies on the behavior and migratory capacity of the Mediterranean monk seal are extremely rare due to the difficulty in performing tagging and tracking experiments [29]. However, observations carried out in different areas in Greece have highlighted the ability of sub-adults and adults to cover a total distance of 100–300 km, with a daily mean of 10–40 km [29]. Moreover, juvenile individuals have also been observed in areas hundreds of kilometers away from their putative origin [23,30,31]. One juvenile female of approximately six months old was found dead in a fishing net along the Libyan coast. The genetic analysis carried out showed a probable origin in the Eastern Mediterranean Sea, suggesting that the young individual may have travelled hundreds of kilometers before being captured [31]. The observation of the haplotype MM03 in the studied specimen and the distance of this area from the Apulian coast (ca. 320 km), allows us to hypothesise the most probable origin of the individual analyzed to be from the Greek islands in the Ionian Sea. However, we cannot exclude the possibility that it was born in Apulia.

The morphological features and the small size of the *M. monachus* individual suggest that it was a very young specimen. Even if the standard length of 118 cm places the pup in age class category three which includes 1–1.7-month-old individuals [32], the morphology allows us to classify it in age class category four (2–4 month old pups) [32]. The monk seal seems to have completed the molt, so it had shed the "lanugo" (a dark woolly coat typical of newborns), and presented a grey color on its back and a pale belly [32,33]. The estimated age of about 2–4 months could suggest that the pup was born along the Apulian coast in the autumn. This hypothesis is supported by the presence in this area of several habitats suitable for the reproduction of monk seals [34] and by the fact that at only 2–4 months of age the pup would still be breastfed by the mother. Mediterranean monk seal females give birth to only one pup per year using coastal caves as shelter. Parturition usually occurs from September to November [6–8] and the pup is breastfed up to five months of age, after which it begins to move and feed itself [35,36]. The great ability of monk seals to travel long distances in the open sea and the genetic results probably indicate that the mother of the pup was a vagrant individual coming from the Ionian Islands (Greece) who chose the Apulian coasts for parturition.

In conclusion, the analysis performed in this study shows that the Mediterranean monk seal pup found along the Apulian coast was probably born in this area and that its mother came from the Greek islands in the Ionian Sea. An origin from the Ionian Islands has been hypothesized as they host the only reproductively active population of monk seals within the Adriatic-Ionian region. Other areas of the Adriatic Sea are only sporadically frequented by individuals of this species. The finding of a Mediterranean monk seal born along the Apulian coast is an important event that proves the recovery of this species in the Mediterranean basin and the probable recolonisation of areas from which it had disappeared. The phenomenon of recolonization has already been hypothesized taking into consideration the increase in sightings in areas of the Mediterranean Sea where the species is not a stable resident [20]. For example, along the Lebanese coast a total of 47 sightings of Mediterranean monk seals was recorded from 2004 to April 2020. These numerous and recent sightings could indicate the presence of remaining individuals from an ancient population or could suggest a probable recolonisation of the Lebanese coast by individuals from the closest reproductive populations of Turkey and Cyprus, indicating an attempt to expand their distribution range [37]. On the other hand, the lack of knowledge about the life cycle, behavior, and real distribution of the species highlights the need to intensify research and monitoring activities. Gathering new information on *M. monachus* will allow us to evaluate the current distribution of the monk seals within the Mediterranean Sea and to understand if there are suitable habitats for the recolonisation of the species and the establishment of new reproductive colonies. In addition, in areas where *M. monachus* populations are already present and in those suitable for recolonisation, fishing activities could be well regulated and the degree of anthropization and coastal habitat deterioration could be reduced. The development of appropriate management plans will be therefore useful to reduce the threats to the species and to promote the recovery and conservation of Mediterranean monk seal within its original distribution range.

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References

1. Scheel, D.M.; Slater, G.J.; Kolokotronis, S.O.; Potter, C.W.; Rotstein, D.S.; Tsangaras, K.; Greenwood, A.D.; Helgen, K.M. Biogeography and taxonomy of extinct and endangered monk seals illuminated by ancient DNA and skull morphology. *Zookeys* **2014**, *409*, 1–33. [CrossRef]
2. Karamanlidis, A.A.; Dendrinos, P. *Monachus monachus. IUCN Red List Threat. Species 2015*; International Union for Conservation of Nature and Natural Resources: Gland, Switzerland, 2015. [CrossRef]
3. Karamanlidis, A.A.; Dendrinos, P.; de Larrinoa, P.F.; Gücü, A.C.; Johnson, W.M.; Kiraç, C.O.; Pires, R. The Mediterranean monk seal *Monachus monachus*: Status, biology, threats, and conservation priorities. *Mamm. Rev.* **2016**, *46*, 92–105. [CrossRef]
4. Johnson, W.M. Monk seals in post-classical history. The role of the Mediterranean monk seal (*Monachus monachus*) in European history and culture, from the fall of Rome to the 20th century. In *Mededelingen No. 39*; Netherlands Commission for International Nature Protection: Leiden, The Netherlands, 2004; pp. 1–91.
5. Johnson, W.M.; Lavigne, D.M. Monk seals in antiquity. The Mediterranean monk seal (*Monachus monachus*) in ancient history and literature. In *Mededelingen No. 35*; Netherlands Commission for International Nature Protection: Leiden, The Netherlands, 1999.
6. Pastor, T.; Aguilar, A. Reproductive cycle of the female Mediterranean monk seal in the Western Sahara. *Mar. Mamm. Sci.* **2003**, *19*, 318–330. [CrossRef]
7. Gucu, A.C.; Gucu, G.; Orek, H. Habitat use and preliminary demographic evaluation of the critically endangered Mediterranean monk seal (*Monachus monachus*) in the Cilician Basin (Eastern Mediterranean). *Biol. Conserv.* **2004**, *116*, 417–431. [CrossRef]
8. Karamanlidis, A.A.; Paravas, V.; Trillmich, F.; Dendrinos, P. First observations of parturition and postpartum behavior in the Mediterranean monk seal (*Monachus monachus*) in the eastern Mediterranean. *Aquat. Mamm.* **2010**, *36*, 27–32. [CrossRef]
9. Gazo, M.; Layna, J.F.; Aparicio, F.; Cedenilla, M.A.; González, L.M.; Aguilar, A. Pupping season, perinatal sex ratio and natality rates of the Mediterranean monk seal (*Monachus monachus*) from the Cabo Blanco colony. *J. Zool.* **1999**, *249*, 393–401. [CrossRef]
10. Stringer, C.B.; Finlayson, J.C.; Barton, R.N.E.; Fernández-Jalvo, Y.; Cáceres, I.; Sabin, R.C.; Rhodes, E.J.; Currant, A.P.; Rodríguez-Vidal, J.; Giles-Pacheco, F.; et al. Neanderthal exploitation of marine mammals in Gibraltar. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14319–14324. [CrossRef] [PubMed]
11. Morales-Pérez, J.V.; Pérez Ripoll, M.; Jordá Pardo, J.F.; Álvarez-Fernández, E.; Maestro González, A.; Aura Tortosa, J.E. Mediterranean monk seal hunting in the regional Epipalaeolithic of Southern Iberia. A study of the Nerja Cave site (Málaga, Spain). *Quat. Int.* **2019**, *515*, 80–91. [CrossRef]
12. Androukaki, E.; Adamantopoulou, S.; Dendrinos, P.; Tounta, E.; Kotomatas, S. Causes of mortality in the Mediterranean monk seal (*Monachus monachus*) in Greece. *Contrib. Zool. Ecol. East. Mediterr. Reg.* **1999**, *1*, 405–411.
13. Karamanlidis, A.A.; Androukaki, E.; Adamantopoulou, S.; Chatzispyrou, A.; Johnson, W.M.; Kotomatas, S.; Papadopoulos, A.; Paravas, V.; Paximadis, G.; Pires, R.; et al. Assessing accidental entanglement as a threat to the Mediterranean monk seal *Monachus monachus*. *Endanger. Species Res.* **2008**, *5*, 205–213. [CrossRef]
14. Osterhaus, A.; Van De Bildt, M.; Vedder, L.; Martina, B.; Niesters, H.; Vos, J.; Van Egmond, H.; Liem, D.; Baumann, R.; Androukaki, E.; et al. Monk seal mortality: Virus or toxin? *Vaccine* **1998**, *16*, 979–981. [CrossRef]
15. Reyero, M.; Cacho, E.; Martínez, A.; Vázquez, J.; Marina, A.; Fraga, S.; Franco, D.J.M. Evidence of saxitoxin derivatives as causative agents in the 1997 mass mortality of monk seals in the Cape Blanc peninsula. *Nat. Toxins* **1999**, *7*, 311–315. [CrossRef]
16. Pastor, T.; Garza, J.C.; Allen, P.; Amos, W.; Aguilar, A. Low genetic variability in the highly endangered mediterranean monk seal. *J. Hered.* **2004**, *95*, 291–300. [CrossRef] [PubMed]
17. Pastor, T.; Garza, J.C.; Aguilar, A.; Tounta, E.; Androukaki, E. Genetic diversity and differentiation between the two remaining populations of the critically endangered Mediterranean monk seal. *Anim. Conserv.* **2007**, *10*, 461–469. [CrossRef]
18. Karamanlidis, A.A.; Gaughran, S.; Aguilar, A.; Dendrinos, P.; Huber, D.; Pires, R.; Schultz, J.; Skrbinšek, T.; Amato, G. Shaping species conservation strategies using mtDNA analysis: The case of the elusive Mediterranean monk seal (*Monachus monachus*). *Biol. Conserv.* **2016**, *193*, 71–79. [CrossRef]

19. Gaubert, P.; Justy, F.; Mo, G.; Aguilar, A.; Danyer, E.; Borrell, A.; Dendrinos, P.; Öztürk, B.; Improta, R.; Tonay, A.M.; et al. Insights from 180 years of mitochondrial variability in the endangered Mediterranean monk seal (*Monachus monachus*). *Mar. Mamm. Sci.* **2019**, *35*, 1489–1511. [CrossRef]
20. Bundone, L.; Panou, A.; Molinaroli, E. On sightings of (vagrant?) monk seals, *Monachus monachus*, in the Mediterranean Basin and their importance for the conservation of the species. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2019**, *29*, 554–563. [CrossRef]
21. Cassoli, P.F.; Fiore, I.; Tagliacozzo, A. Butchery and exploitation of large mammals in the Epigravettian levels of Grotta Romanelli. *Anthropozoologica* **1997**, *25*, 309–318.
22. Di Turo, P. Presenza della foca monaca (*Monachus monachus*) nell'area mediterranea con particolare riferimento alla Puglia. *Thalassia Salentina* **1984**, *14*, 66–84. [CrossRef]
23. Mo, G. Mediterranean monk seal (*Monachus monachus*) sightings in Italy (1998–2010) and implications for conservation. *Aquat. Mamm.* **2011**, *37*, 236–240. [CrossRef]
24. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; Mcgettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [CrossRef] [PubMed]
25. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
26. Bandelt, H.-J.; Forster, P.; Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. [CrossRef] [PubMed]
27. Panou, A.; Jacobs, J.; Panos, D. The endangered mediterranean monk seal *Monachus monachus* in the Ionian Sea, Greece. *Biol. Conserv.* **1993**, *64*, 129–140. [CrossRef]
28. Panou, A. Monk seal sightings in the central Ionian Sea. A network of fishermen for the protection of the marine resources. In Proceedings of the European Cetacean Society Annual Conference, Istanbul, Turkey, 28 February 2009; p. 6.
29. Adamantopoulou, S.; Androukaki, E.; Dendrinos, P.; Kotomatas, S.; Paravas, V.; Psaradellis, M.; Tounta, E.; Karamanlidis, A.A. Movements of Mediterranean monk seals (*Monachus monachus*) in the Eastern Mediterranean Sea. *Aquat. Mamm.* **2011**, *37*, 256–261. [CrossRef]
30. Bayed, A. Further observations of Mediterranean monk seals on the north Atlantic coast of Morocco. *Monachus Guard.* **2001**, *4*, 45–47.
31. Alfaghi, I.A.; Abed, A.S.; Dendrinos, P.; Psaradellis, M.; Karamanlidis, A.A. First confirmed sighting of the mediterranean monk seal (*Monachus monachus*) in Libya since 1972. *Aquat. Mamm.* **2013**, *39*, 81–84. [CrossRef]
32. Mo, G.; Zotti, A.; Agnesi, S.; Finola, M.G.; Bernardini, D.; Cozzi, B. Age classes and sex differences in the skull of the Mediterranean monk seal, *Monachus monachus* (Hermann, 1779). A study based on bone shape and density. *Anat. Rec.* **2009**, *292*, 544–556. [CrossRef]
33. Samaranch, R.; González, L.M. Changes in morphology with age in Mediterranean monk seals (*Monachus monachus*). *Mar. Mamm. Sci.* **2000**, *16*, 141–157. [CrossRef]
34. Bundone, L.; Fai, S.; D'Ambrosio, P.; Onorato, R.; Minonne, F.; Molinaroli, E. Coastal habitat availability for the Mediterranean monk seal, *Monachus monachus* (Hermann, 1779), in Salento: Preliminary results. *Biol. Mar. Mediterr.* **2014**, *21*, 253–254.
35. Aguilar, A.; Cappozzo, L.H.; Gazo, M.; Pastor, T.; Forcada, J.; Grau, E. Lactation and mother-pup behaviour in the Mediterranean monk seal *Monachus monachus*: An unusual pattern for a phocid. *J. Mar. Biol. Assoc. UK* **2007**, *87*, 93–99. [CrossRef]
36. Kıraç, C.O.; Ok, M. Diet of a Mediterranean monk seal *Monachus monachus* in a transitional post-weaning phase and its implications for the conservation of the species. *Endanger. Species Res.* **2019**, *39*, 315–320. [CrossRef]
37. SPA/RAC-UNEP/MAP. *On the Occurrence of the Mediterranean Monk Seal* Monachus monachus *(Hermann, 1779) in the Lebanese Waters (Eastern Mediterranean Sea)*; Badreddine, A., Limam, A., Ben-Nakhla, L., Eds.; SPA/RAC: Tunis, Tunisia, 2020; p. 12.