First report of the scavenging isopod Natatolana neglecta (Crustacea: Isopoda: Cirolanidae) feeding on a sea turtle

REVUELTA OHIANA
Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia

DOMÈNECH FRANCESC
Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia

KEABLE STEPHEN
Australian Museum Research Institute, 1 William Street Sydney NSW 2010 Australia.

MÍGUEZ-LOZANO RAÚL
Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia

https://doi.org/10.12681/mms.19058

To cite this article:
REVUELTA, O., DOMÈNECH, F., KEABLE, S., & MÍGUEZ-LOZANO, R. (2019). First report of the scavenging isopod Natatolana neglecta (Crustacea: Isopoda: Cirolanidae) feeding on a sea turtle. Mediterranean Marine Science, 20(2), 297-301. doi:https://doi.org/10.12681/mms.19058
First report of the scavenging isopod *Natatolana neglecta* (Crustacea: Isopoda: Cirolanidae) feeding on a sea turtle

Ohiana REVUELTA¹, Francesc DOMÈNECH¹, Stephen KEABLE² and Raúl MÍGUEZ-LOZANO³

¹Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, 46980 Paterna, Valencia, Spain
²Australian Museum Research Institute, 1 William Street, Sydney, NSW 2010, Australia

Corresponding author: ohiana.revuelta@uv.es.

Handling Editor: Kostas KAPIRIS

Received: 22 January 2019; Accepted: 16 March 2019; Published on line: 28 May 2019

Abstract

A juvenile male loggerhead sea turtle (*Caretta caretta*) was found dead in April 2015, entangled in a trammel net on the Mediterranean coast of Spain. Post-mortem examination revealed the presence of ninety-five isopods dispersed in the coelomic cavity, and inside the oesophagus and skull. All individuals found scavenging on the sea turtle were identified as *Natatolana neglecta* (Hansen, 1890) (Isopoda: Cirolanidae). Genetic analysis of the isopod gut contents showed that they were feeding on turtle tissue, confirming that *N. neglecta* can also attack dead sea turtles. This study shows the value of cirolanids as potential indicators of the cause of death in stranded sea turtles.

Keywords: Bycatch; *Caretta caretta*; Isopod; Mediterranean; *Natatolana neglecta*; Scavenger.

Introduction

Isopods of the family Cirolanidae are distributed worldwide, including marine, estuarine and some freshwater environments (Bruce *et al.*, 2002). Some cirolanids are micro-predators on the surface of fishes or invertebrates (Bruce, 1986; Brusca *et al.*, 1995), other species are scavengers on marine fauna with no apparent preference for particular carrion types (Stepien & Brusca, 1985; Bruce, 1986; Berrow, 1994; Keable, 1995; Wong & Moore, 1995; Marsden, 1999). For some species it is not clear if they are only scavengers or also predators (Bunkley-Williams & Williams, 1998). Observations on cirolanids associated with sea turtles are apparently frequent, but the species are seldom identified or reported (Williams *et al.*, 1996). There are records of cirolanids from loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*) and green (*Chelonia mydas*) sea turtles (Bus tard, 1976; Williams *et al.*, 1996), all of them are cases of specimens located on the exterior of the turtles. Association of cirolanids with sea turtles has been considered to result from opportunistic behavior, particularly to turtles injured in nets or resting on the sea floor (Williams *et al.*, 1996). However, to the best of our knowledge, none of these hypotheses have been confirmed.

In this study we report a swarming attack by isopods on a dead loggerhead sea turtle and identification of the isopods based on morphological and molecular methods. The nature of interaction of the isopods with the turtle, and the value of cirolanids as potential indicators of the cause of death in stranded turtles, is also discussed.

Material and Methods

In April 2015, a juvenile male loggerhead sea turtle (curved carapace length notch to tip = 34.8 cm) was found dead, entangled in a trammel net in Vinaros (40.466667° N, 0.466667° W), Valencian Community, Mediterranean coast of Spain (Fig. 1A). During necropsy, the oesophagus, stomach, and intestinal tract were removed from the body cavity and all organs were examined for lesions and parasites. Isopods were found inside the oesophagus and dispersed in the coelomic cavity (Fig. 1B-C).

The isopods were identified morphologically based on the descriptions, diagnoses and keys of Bruce (1981), Bruce (1986), Keable & Bruce (1997) and Keable (2006). Observations on cirolanids associated with sea turtles are apparently frequent, but the species are seldom identified or reported (Williams *et al.*, 1996). There are records of cirolanids from loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*) and green (*Chelonia mydas*) sea turtles (Bus tard, 1976; Williams *et al.*, 1996), all of them are cases of specimens located on the exterior of the turtles. Association of cirolanids with sea turtles has been considered to result from opportunistic behavior, particularly to turtles injured in nets or resting on the sea floor (Williams *et al.*, 1996). However, to the best of our knowledge, none of these hypotheses have been confirmed.

In this study we report a swarming attack by isopods on a dead loggerhead sea turtle and identification of the isopods using DNA barcoding. For this analysis, 10 individuals were used to extract the DNA using a Qiagen DNeasy® Blood & Tissue kit following the manufacturer’s instructions (Qiagen, Germany). To compare with other sequenced isopods, cytochrome c oxidase subunit 1 (CO1) was amplified using primers LCO1490 and HCO2198 from Folmer *et al.* (1994).
A further analysis utilising DNA barcoding was performed to confirm that the gut contents found in two of the isopods was blood and tissue of the turtle. These contents were removed from the isopods, and their DNA extracted with the same technique described above. To compare with sequences available at GenBank the R35 intron was sequenced using the primers R35Ex1 and R35Ex2 from Fujita et al. (2004). PCR amplifications were performed with the ready-to-use MyFi Mix (Bioline Ltd., United Kingdom).

The thermocycling profiles were applied following Raupach et al. (2015) for CO1, and Fujita et al. (2004) for the R35 intron. PCR amplicons were purified using a Macherey-Nagel NucleoSpin® Gel and PCR Clean-Up kit (Macherey-Nagel, Germany), and PCR primers were used for sequencing. Sequencing was performed by the commercial sequence provider Macrogen (Netherlands). Contigs were assembled using BioEdit v.7.2.5 (Hall, 1999). Consensus sequences of CO1 regions were submitted to GenBank (accession numbers MH509756, MH509757, MH509758). A BLAST (Basic Local Alignment Search Tool) was used in order to try to determine the species of isopod, and/or the more similar ones.

The new generated sequences of CO1 were aligned with those of other isopods, including Natatolana spp., recorded by Raupach et al. (2015) using MAFFT ver. 7 software (Katoh & Standley, 2013), and revised in MEGA 6.0 (Tamura et al., 2013). The brachyuran crustacean Cancer pagurus was included as an outgroup (Raupach et al., 2015). The total alignment comprised 25 sequences and 645 nucleotide positions. The nucleotide substitution model was estimated using jModelTest ver. 2.1.6 (Darriba et al., 2012). Based on the Akaike information criterion (AIC), the model GTR+F (general-time-reversible model with gamma distributed among-site rate variation) was found to be the best fitting the dataset.

Phylogenetic analyses of CO1 sequences were performed using Bayesian Inference (BI) and Maximum Likelihood (ML) criteria. ML analyses were performed using PhyML ver. 3.0 software (Guindon et al., 2010). Branch support was estimated by bootstrap analysis with 1000 replicates. BI analyses were performed using MrBayes ver. 3.2 (Ronquist et al., 2012) with four Markov Chain Monte Carlo (MCMC) chains run for one million generations with a sampling frequency of 500 and a “burn-in” value of 25% of the stored trees. A majority rule consensus tree was built after discarding the first 25% of the trees. The nodal support was estimated as posterior probabilities (PP) (Huelsenbeck et al., 2001).

Voucher specimens of the isopods are deposited in the Australian Museum, Sydney, Australia, registration number P.101627.

**Fig. 1:** Isopods of the species *Natatolana neglecta* scavenging on a loggerhead sea turtle *Caretta caretta*. A: juvenile loggerhead stranded in Spain (western Mediterranean). B: *N. neglecta* individual found in the coelomic cavity of the turtle. C: Detail of the holes (arrows) observed in the oesophagus wall. D: specimen found feeding on the turtle’s liver. E: specimen found inside the skull, feeding on the turtle’s salt gland. Scale bars 10 cm (A) and 1 cm (B, C and E) and 2 cm (D).
Results and Discussion

Ninety-five individual isopods were found in the turtle carcass during the necropsy. All of the specimens were found inside the turtle, dispersed in the coelomic cavity, on the external surface of organs, glands and tissues. The examination of the turtle’s organs showed several holes in the oesophagus wall, which were probably made by mechanical penetration of the isopods to reach the coelomic cavity (Fig. 1C). Feeding activity was also observed on the turtle’s liver (Fig. 1D). Individuals were also found inside the skull, where they had presumably eaten the salt gland of the right side (Fig. 1E). A hole found in the right eye of the turtle could be the entry route followed by the isopods, at least for the ones found in the skull. Other possible points of access could be the mouth and nose.

Specimens agreed with the morphological diagnostic and descriptive generic characters of *Natatolana* provided by Bruce (1981), Bruce (1986), Brusca et al. (1995) and Keable (2006), including the setation of pereopod 7, the short antennule and details of the frontal lamina that Keable (2006) highlighted as particularly distinctive. Out of the six species of the genus *Natatolana* reported in the North Atlantic and Mediterranean, the specimens were identified as *Natatolana neglecta* (Hansen, 1890) based on the keys, descriptions and diagnosis provided by Keable & Bruce (1997) and Keable (2006). The pleotelson shape (anterolateral margins almost straight and angling posteriorly toward the midline; posterolateral margins straight, markedly angled to anterolateral margins and meeting at an obtuse angle), with a high number (10 to 14) of robust setae on the margins, is especially characteristic. This coupled with the well-developed eyes, lack of robust setae on the propodal palm of pereopods two and three (although single setae were found on these pereopods in a few individuals) and relatively narrow basis of pereopod 7 (distance between the anterior margin and medial carina less than between posterior margin and medial carina – following the orientation indicated in Keable (2006) fig. 3B) are sufficient to separate the material examined from other species of *Natatolana* that are known from the area and also from similar species occurring elsewhere.

The BLAST tool showed a close similarity of our species to the other three species of the genus *Natatolana* (*N. borealis, N. gallica* and *N. rossi*) available at GenBank, and the phylogenetic tree obtained (Fig. 2) shows a distinct clade formed by the four species of *Natatolana*. This supports the identification of our specimens as a species of *Natatolana*. The results of the analysis of the R35 intron from the blood and tissue remains found in the gut of some of the specimens, confirm that they belonged to a loggerhead sea turtle.

Whether the turtle was scavenged alive or already dead in the net is unknown, but *Natatolana* species are opportunistic and voracious scavengers (references summarized in Keable (2006)), and they usually swarm in vast numbers and attack damaged fish, particularly at dusk or at night (Wong & Moore, 1995; Stepien & Brusca, 1985). They can cause damage to the commercial fishing operations (Bird, 1981; Stepien & Brusca, 1985; Berrow, 1994; Johansen & Brattegard, 1998; Poore & Bruce, 2012) and have been described feeding on dying fishes trapped in trammel nets and long-line fisheries, including a case reported in Italy and involving *N. neglecta* (Mizzan, 1995). Local fishermen in the study area have also reported the presence of scavenging isopods on fishes trapped in trammel nets (authors, pers. obs.). Hence, the association of

![Fig. 2: Phylogenetic tree of Isopoda spp. derived from Bayesian Inference and Maximum Likelihood analysis using CO1 region.](http://epublishing.ekt.gr)
**Natatolana** specimens with this entangled turtle is consistent with the ecology and feeding pattern of these cirolanids (Johanssen & Brattegard, 1998). Soak time of the net in which the necropsied turtle was found was eight hours between 20:00 hr to 06:00 hr; the turtle may have been entangled for several hours and isopods started to scavenge when the turtle was dead or near dead, without movement.

Sea turtle strandings have been used to identify mortality causes, allowing inferences on patterns of bycatch (Panagopoulos *et al.*, 2003; Witt *et al.*, 2007). However, determining a specific cause of death is not possible for most of the stranded turtles, due to the absence of external evidence or the degree of decomposition. A common procedure to diagnose the death as due to bycatch uses several criteria [e.g. presence of lines of nylon wrapped around their necks and flippers (Blasi *et al.*, 2016); imbedded fishing hook (Parga, 2012); gas bubbles within tissues (Fahlman *et al.*, 2017)]. Because presence of cirolanid isopods has been used in forensic assessment of cadavers retrieved from the sea (Nagano, 1963; Colombo & Telisinghe, 2010; Tiememsna *et al.*, 2017), occurrences of scavenging isopods in sea turtle carcasses, or the signals of their activity described here, could be added as further important evidence in examination protocols to diagnose instances of bycatch in trammel nets involving sea turtles in future necropsies.

**Acknowledgements**

This study was possible thanks to the financial support of the project Prometeo II (2015-018) of the Generalitat Valenciana. We acknowledge the support of the Conselleria d’Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural de la Generalitat Valenciana. We are grateful to the staff of the Marine Zoology Unit of the Universitat de Valencia for the technical assistance during the necropsy and to the Fundación Oceanogràfic de Valencia for their participation in the regional sea turtle stranding network. We want to thank Jose Luis Crespo-Picazo from Fundación Oceanogràfic for some of the photographs from fig. 1. FD is supported by a predoctoral grant UV-INV PREDOC15-265927 of the Universitat de Valencia.

**References**

Berrow, S., 1994. Fish predation by the marine crustaceans *Orchomene nana* and *Natatolana borealis*. *The Irish Naturalists’ Journal*, 24, 514.

Bird, P.M., 1981. The occurrence of *Cirolana borealis* (Isopoda) in the hearts of sharks from Atlantic coastal waters of Florida. *Fishery Bulletin*, 79 (2), 376-383.

Blasi, M.F., Roscioni, F., Matte, D., 2016. Interaction of loggerhead turtles (*Caretta caretta*) with traditional fish aggregating devices (FADs) in the Mediterranean Sea. *Herpetological Conservation and Biology*, 11, 386-401.

Bruce, N.L., 1981. Cirolanidae (Crustacea: Isopoda) of Australia: Diagnoses of *Cirolana* Leach, *Metacirolana* Nierstrasz, *Necocirolana* Hale, *Anopsilana* Paulian & Deboutteville, and three new genera – *Natatolana*, *Poliolana* and *Carte-

Bruce, N.L., 1986. Cirolanidae (Crustacea: Isopoda) of Australia. *Records of the Australian Museum Supplement*, 6, 1-239.

Bruce, N.L., Lew Ton, H.M., Poore, G.C.B., 2002. Cirolanidae Dana, 1852. In: Houston WWK, Beesley P.L. (Eds) Zoological Catalogue of Australia: *Crustacea*: Malacostraca: *Syncarida*, *Peracarida*: *Isopoda*, *Tanaidacea*, *Mictace*, *Thoracata* et *Decapoda*. *Fauna of Australia* series, 3. CSIRO Publishing, Melbourne, Australia, pp. 138-157.

Bruce, R.C., Wetzner, R., France, S.C., 1995. Cirolanidae (Crustacea: Isopoda: Flabellifera) of the Tropical Eastern Pacific. *Proceedings of the San Diego Society of Natural History*, 30, 1-96.

Bunkley-Williams, L., Williams, E.H. Jr., 1998. Isopods associated with fishes: A synopsis and corrections. *Journal of Parasitology*, 84 (5), 893-896.

Bustard, H.R., 1976. Turtles of coral reefs and coral islands. In: *Jones OA, Endean R (Eds) Biology and geology of coral reefs* 3, 343-368. *Academic Press, New York*.

Colombo, S.M., Telisinghe, P.U., 2010. An unusual finding in a body recovered from the sea. *Journal of Forensic and Legal Medicine*, 17 (5), 289-90.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9 (8), 772.

Fahlman, A., Crespo-Picazo, J.L., Sterba-Boatwright, B., Stacy, B.A., García-Párraga, D., 2017. Defining risk variables causing gas embolism in loggerhead sea turtles (*Caretta caretta*) caught in trawls and gillnets. *Scientific Reports*, 7, 2739.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, 3 (5), 294-299.

Fujita, M.K., Engstrom, T.N., Starkey, D.E., Shaffer, H.B., 2004. Turtle phylogeny: Insights from a novel nuclear intron. *Molecular Phylogenetics and Evolution*, 31 (3), 1031-1040.

Guindon, S., DuFayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. *et al.*, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, 59 (3), 307-321.

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.

Hansen, H.J., 1890. Cirolanidae et familie nonnullae pro-pinquae Musei Hauennisis. Et Bidrag til Kunsten som nogle Næg til isope Brexys. Kongelige Danske Videnskabernes Selskabs Skrifter, 6te Række. *Naturvidenskabelig og Matematisk Afdeling*, 3, 239-426.

Hauelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294 (5550), 2310-2314.

Johanssen, P.O., Brattegard, T., 1998. Observations on behaviour and distribution of *Natatolana borealis* (lilljeborg) (*Crustacea*, *Isopoda*). *Sarsia*, 83 (4), 347-360.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hansen, H.J., 1890. Cirolanidae et familie nonnullae pro-pinquae Musei Hauennisis. Et Bidrag til Kunsten som nogle Næg til isope Brexys. Kongelige Danske Videnskabernes Selskabs Skrifter, 6te Række. *Naturvidenskabelig og Matematisk Afdeling*, 3, 239-426.

Hauelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294 (5550), 2310-2314.

Johanssen, P.O., Brattegard, T., 1998. Observations on behaviour and distribution of *Natatolana borealis* (lilljeborg) (*Crustacea*, *Isopoda*). *Sarsia*, 83 (4), 347-360.
alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30 (4), 772-780.

Keable, S.J., 1995. Structure of the marine invertebrate scavenging guild of a tropical reef ecosystem: field studies at Lizard Island, Queensland, Australia. *Journal of Natural History*, 29 (1), 27-45.

Keable, S.J., 2006. Taxonomic Revision of *Natatolana* (Crustacea: Isopoda: Cirolanidae). *Records of the Australian Museum*, 58 (2), 133-244.

Keable, S.J., Bruce, N.L., 1997. Redescription of the north Atlantic and Mediterranean species of *Natatolana* (Crustacea: Isopoda: Cirolanidae). *Journal of the Marine Biological Association of the United Kingdom*, 77 (3), 655-706.

Marsden, I.D., 1999. Feeding, respiration, and aerial exposure in a scavenging cirolanid isopod from New Zealand. *Journal of Crustacean Biology*, 19, 459-466.

Mizzan, L., 1995. *Cirolana cfr. neglecta* Hansen, 1890 (Crustacea, Isopoda, Cirolanidae) nelle coste del Veneziano: note su di un attacco ad una postazione di pesca. *Bollettino del Museo civico di storia naturale di Venezia*, 44, 145-151.

Nagano, T., Kawakami, K., Kajiyura, Y., Sakaguchi, M., Yamamoto, H., 1963. Rapid destruction of submerged cadavers by tiny marine animals. *Wakayama Medical Reports*, 8, 31-39.

Panagopoulos, D., Sofouli, E., Teneketsiz, K., Margaritoulis, M., 2003. Stranding data as an indicator of fisheries induced mortality of sea turtles in Greece. In: Margaritoulis D, Demetrioupolous A (Eds) Proceedings of the First Mediterranean Conference on Marine Turtles, Rome, 24-28 October 2001. Barcelona Convention–Bern Convention–Bonn Convention (CMS), Nicosia, pp. 202-206.

Parga, M.L., 2012. Hooks and sea turtles: a veterinarian’s perspective. *Bulletin of Marine Science*, 88 (3), 731-741.

Poore, G.C.B., Bruce, N.L., 2012. Global Diversity of Marine Isopods (Except Asellota and Crustacean Symbionts). *PLoS ONE* 7: e43529.

Rausch, M.J., Barco, A., Steinke, D., Beermann, J., Laakmann, S. et al., 2015. The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. *PLoS ONE* 10:e0139421.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A. et al., 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology*, 61 (3), 539-542.

Stepien, C.A., Brusca, R.C., 1985. Nocturnal attacks on nearshore fishes in southern California by crustacean zooplankton. *Marine Ecology Progress Series*, 25, 91-105.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30 (12), 2725-2729.

Tiemensma, M., Bruce, N.L., Willan, R.C., 2017. Post-mortem human cadaver scavenging by marine crustaceans (Isopoda: Cirolanidae) in tropical waters. *Forensic Science, Medicine, and Pathology*, 13, 515-517.

Williams, E.H., Bunkley-Williams, L., Boulon, R.H., Eckert, K.L., Bruce, N.L., 1996. *Excorallana acuticaudata* (Isopoda, Corallanidae) an Associate of Leatherback Turtles in the Northeastern Caribbean, with a Summary of Isopods Recorded from Sea Turtles. *Crustacea*, 69 (8), 1014-1017.

Witt, M.J., Broderick, A.C., Johns, D.J., Martin, C., Penrose, R. et al., 2007. Prey landscapes help identify potential foraging habitats for leatherback turtles in the northeast Atlantic. *Marine Ecology Progress Series*, 337, 231-244.

Wong, Y.M., Moore, P.G., 1995. Biology of feeding in the scavenging isopod *Natatolana borealis* (Isopoda: Cirolanidae). *Ophelia*, 43 (3), 181-19.