Tissue and Cell 45 (2013) 321–327

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Iridovirus infection in terrestrial isopods from Sicily (Italy)

Pietro Lupetti[a], Giuseppe Montesanto[b,*], Silvia Ciolfi[a], Laura Marri[a], Mariangela Gentile[a], Eugenio Paccagnini[a], Bianca Maria Lombardo[b]

[a] Dipartimento di Scienze della Vita, Università di Siena, Siena, Italy
[b] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Catania, Catania, Italy

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 2 May 2013
Accepted 2 May 2013
Available online 10 June 2013

Keywords:
Iridovirus
Crustacea
Isopoda
Oniscidea
Infection
Electron microscopy
DNA sequencing

ABSTRACT

During our researches on systematics and ecology of terrestrial isopods, carried out in western Sicily, some specimens showing a blue–purple coloration were collected; they belonged to four species: *Armadillidium decorum* Brandt, 1833, *Trichoniscus panormidensis* Montesanto et al., 2011, *Philoscia affinis* Verhoeff, 1908, *Porcellio siculoccidentalis* Viglianisi et al., 1992. We hypothesized that such coloration could be due, as reported in literature, to characteristic paracrystalline arrays of virions inside the tissues of blue colored specimens. Ultrastructural observations by transmission electron microscopy, on tissues of *A. decorum*, showed the presence of electron-dense viral particles, with a diameter of nearly 0.12 μm. Dual-axis tomography, performed on specimens of *A. decorum*, evidenced an icosahedral structure of viral particles matching with that of Isopod Iridescent Virus (IIV). Molecular analysis, on 254 bp portion of the major capsid protein (MCP) gene, allowed to place the virus into IIV-31 group, already known for other oniscidean species. The symptoms of infected individuals and the course of the disease were followed in laboratory, indicating similarities with other studies on Isopod Iridoviruses. Moreover, some notes on reproduction of infected ovigerous females are reported. Our data support unequivocal and direct evidences for the first case of IIV infection in terrestrial isopods reported in Italy.

1. Introduction

The type-species of Iridovirus was firstly isolated by Xeros (1954) from the crane fly *Tipula paludosa* Meigen 1835. Afterwards, different Iridoviruses have been recorded for other Insects, mainly Diptera, Coleoptera and Lepidoptera (Kelly and Robertson, 1973; Carey et al., 1978). In the terrestrial isopods, the virus was firstly isolated in 1980 (Federici, 1980; Cole and Morris, 1980) and named Isopod Iridescent Iridovirus (IIV). The most evident feature of the infection is a clear blue–purple coloration, distributed on the body and on the appendages of infected specimens. Nowadays is well-known that the coloration is produced by paracrystalline arrays of virions inside the parasitized cells (Federici, 1984). According to Federici (1984) the presence of purple–blue color can be taken as a quite definitive evidence of Iridovirus infection. Before Iridoviruses were identified, the distinct purple to blue iridescent coloration produced in infected individuals was reported for *Ligidium hypnorum* (Cuvier, 1792) and *Philoscia muscorum* (Scopoli, 1763) in France (Lereboullet, 1843, 1853; Legrand, 1948; Vandel, 1962), and for *Trichoniscus pusillus* (Brandt, 1833) in England (Standen, 1917) and, many other samples of infected Oniscidean were reported from several locations in Europe and North America (Williams, 2008). A complete list of species and "varieties" described in older literature which are certainly infected with Iridovirus, together with a list of terrestrial isopods carrying Iridovirus infection, is reported by Wijnhoven and Berg (1999) and Williams (2008). The authors cited a total of 19 species, including newly reported samples. Recently, eight species of terrestrial isopods from Japan have been found infected with IIV (Karasawa et al., 2012); two of them are new reports of Iridovirus infection.

In a wider context of researches on ecology and systematics of terrestrial isopods, recently carried out on Mt. San Giuliano (Erice) in western Sicily, new species and new records of terrestrial isopods were reported (Montesanto et al., 2011). During those samplings some specimens belonging to four species: *Armadillidium decorum* Brandt, 1833, *Trichoniscus panormidensis* Montesanto et al., 2011, *Philoscia affinis* Verhoeff, 1908, *Porcellio siculoccidentalis* Viglianisi, et al., 1992, have been collected showing a clear blue–purple coloration, distributed on the body and on the appendages.

The aim of this study was verifying the presence of Iridoviridae in terrestrial isopods from Sicily, and to compare the detected Isopod Iridescent Virus (IIV) with other IIV already known for

* Corresponding author. Tel.: +39 095 7306048.
E-mail addresses: g.montesanto@unict.it, gipo.montesanto@gmail.com (G. Montesanto).

0040-8166/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tice.2013.05.001

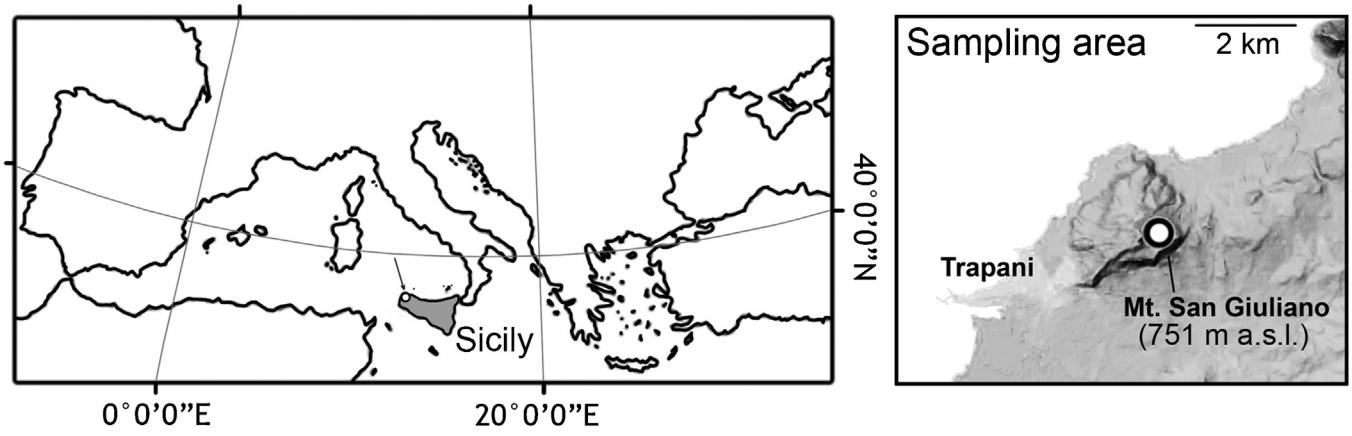

Fig. 1. Sampling site in western Sicily (Italy); WGS84 coordinates: 38°02′13″N, 12°35′35″E.

Oniscidean species but not yet detected in Italy. Another goal of this study was also to briefly describe the symptoms and the course of the disease in lab reared isopods specimens. Moreover, some facts about the biology of reproduction were taken into account, trying to asses a notation by Hess and Poinar (1985): "it would be interesting to determine if these individuals are infected with an Iridovirus and if so, if they are still able to mate and reproduce before being destroyed by the disease. If so, this would represent a remarkable tolerance to the disease".

2. Materials and methods

2.1. Terrestrial isopods sampling and breeding

The animals were collected, using entomological forceps, in the litter and under stones of holm-oak woods at the top of Mount San Giuliano, in Trapani province (western Sicily). Sampling site is indicated in Fig. 1. In laboratory, appropriate breedings were carried out in plastic boxes of 35 cm × 60 cm, with soil taken from the sampling sites and previously sterilized. The specimens were fed with sterilized and rehydrated plate-tree leaves, and with slices of potatoes and carrots. The breeding substrate was periodically moistened by nebulized water, and kept at constant temperature of 20 °C (±1 °C).

2.2. Electron microscopy

Nervous tissue of *A. decorum*, *T. panormidensis*, and *P. siculoccidentalis* dissected in 0.1 M phosphate buffer (PB), pH 7.2, was fixed for 1 h at 4 °C in 2.5% glutaraldehyde in PB. Samples were then rinsed in PB and postfixed for 1 h in 1% osmium tetroxide water solution. Tissues were then rinsed in PB, dehydrated in ascending alcohol series and embedded in Epon-Araldite epoxy resin. Ultrathin sections (60–70 nm) obtained with a Reichert Ultracut IIE ultramicrotome were stained with uranyl acetate and lead citrate according to Reynolds (1963). Thin sections were then observed and imaged by a Philips CM10 transmission electron microscope operating at an electron accelerating voltage of 80 kV. From the resin-embedded samples were also obtained 120–250 nm thick sections; after the above described staining procedure, they were treated on both sides with a solution of colloidal gold particles of 10 nm and examined using a Philips CM 200 FEG TEM equipped with a digital camera (TVIPS TemCam-F224HD). The tomographic series were acquired by a computer assisted routine with the software TVIPS EMMENU4 and EMTool controlling TEM settings, stage movements, magnifications and defocus along image collection. Tomographic series were acquired from regions of the sample that remained visible at least throughout a tilting excursion ranging from +55° to −55°. Images were collected at incremental tilting steps of 1° and with dual axis tilting strategy. Tomograms were generated from dual axis tomographic series of images using the open source software IMOD (http://bio3d.colorado.edu/imod/). All renderings were produced by the open source software Chimera (http://www.cgl.ucsf.edu/chimera/).

2.3. Molecular analysis

Genomic DNA was extracted from blue colored specimens of *A. decorum*, *T. panormidensis*, and *P. siculoccidentalis* using the Wizard® Genomic DNA Purification kit (Promega). To amplify a region of 254 bp of the major capsid protein (MCP) gene, specific primers were designed on the basis of the highly conserved regions of sequences identified in other isopods, especially those from *A. vulgare* and *P. scaber* (GenBank accession numbers: AF042337.1 and AF297060.1, respectively). The primers were: IIVfor: 5′-ATTGGTAACATTTCGGCTCTTATC-3′; IIVrev: 5′-GCACCAACTACAGGTACAACAGAC-3′. About 100 ng of genomic DNA was used in each PCR reaction following the protocol of Webby and Kalmakoff (1998). A contamination control (reaction without template) and a template control (reaction with primers for the actin gene) were set up for each amplification experiment. PCR products were visualized on a 1.5% agarose gel in TAE 1%, and the expected band of 254 bp, corresponding to a portion of the MCP gene, was purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega) and sequenced on both strands (Bio-Fab Research). The sequences have been submitted to GenBank database under the accession numbers: JX847599 (*T. panormidensis*); JX847600 (*A. decorum*); JX847601 (*P. siculoccidentalis*).

2.4. Data analysis

The 254 bp nucleotide and the corresponding deduced amino acid sequences, obtained by PCR from the three Sicilian isopod species, were compared with other MCP gene sequences by means of BLASTn and BLASTp tools at NCBI website (http://www.ncbi.nlm.nih.gov/). Nucleotide and deduced amino acid sequences were aligned using ClustalW with default settings (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic tree was inferred by the Neighbor-Joining (NJ) method, performing a 1000-replicates bootstrap test, using MEGA version 5 (Tamura et al., 2011). The list of Iridoviruses and host species involved in this study, together with sites of isolation and Genbank accession numbers, are reported in Table 1.