

Nematocyst types and venom effects of *Aurelia aurita* and *Velella velella* from the Mediterranean Sea



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ABSTRACT

Natural substances produced by venomous marine organisms are thought to be possible sources of useful compounds and new drugs having the potential to open new ways for pharmacology, nutrition and environmental applications. In this framework, cnidarians are very interesting being widely distributed and all are venomous organisms; so, a deep knowledge of their occurrence, morphology of venomous structures and of effects of venoms at cellular level is fundamental to evaluate the possible utilization of venomous compounds or extracts. In this research, the morphology and occurrence of nematocysts in two cnidarian species (*Aurelia aurita*, *Velella velella*), and the preliminary evaluation of the cytotoxicity of *V. velella* crude extract, of which cytotoxicity on cell cultures at present is unknown, were considered. The specimens were sampled in Güllük Bay, South-western coast of Turkey, and in the Gulf of Genova, Northwestern coast of Italy. Six nematocyst types (a-isorhiza, A-isorhiza, O-isorhiza, eurytele, polyspiras, birhopaloid) having different sizes, were observed in *A. aurita*, and two types (eurytele and stenotele) in *V. velella*. The crude extract from *V. velella* showed cytotoxic activity against cultured fibroblasts L929 at high doses, while inducing cell proliferation at low doses. The protein content in the extract increased remarkably after disruption of nematocysts.

1. Introduction

Cnidarians (phylum Cnidaria) are one of the most widespread taxa around the globe. Their sudden swarms ('blooms' or 'outbreaks') and thus their impact on human coastal and marine activities have been increasing in the last decades both in terms of size and frequency (UNEP, 1984; 1991).

Cnidarians are specialized predators able to sting and paralyze their prey throughout a wide range of venoms; therefore, due to their increasing number beachgoers, holidaymakers, fishermen, and other sea-workers are becoming more exposed and at risk of being stung and poisoning (Boero, 2013; Bonello et al., 2017; Condon et al., 2013; Cushing, 1990). In addition, jellyfish aggregations are also able to clog fishing nets, thus affect fisheries.

Toxic and allergic reactions must be recorded on people after contact with jellyfish (Burnett, 2001; Fenner, 1998). Notably, some jellyfish species cause serious effects on humans and, in general, cnidarian venoms can induce a wide variety of symptoms, ranging from local

inflammation of skin to more severe phenomena with cardiac and respiratory failure, as well as algogenic and neurotoxic symptoms (Benoit, 1998; Diochot et al., 2007; Smith and Blumenthal, 2007; Wanke and Restano-Cassulini, 2007). Cardiovascular and respiratory problems (Burnett, 1991, 2009; Endean, 1981) and even fatal cases leading to death within minutes after the sting were recorded (Brinkman and Burnell, 2009; Tibballs, 2006). Some procedures and methods are available to counteract cnidarian venoms, but the usefulness of some of them is debated (Killi and Mariottini, 2018).

In the Mediterranean Sea, jellyfish are not extremely toxic to humans, but they are potentially dangerous to allergic and sensitive subjects (UNEP, 1984, 1991). Among Mediterranean species, *Physalia physalis*, *Carybdea marsupialis*, *Pelagia noctiluca* and the lessepsian species *Rhopilema nomadica* are known to possess remarkable stinging properties, and are also involved in clogging fishing nets and water intakes of power plants (Mariottini and Pane, 2010).

Jellyfish venoms are known to be a group of biologically active substances, and are supposed to have potentially a wide range of

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applications in applied research (Šuput, 2009). The role of Cnidarian toxins in drug discovery has been widely discussed and studied during the last four decades, since the first studies on venom toxicity were published (Mariscal, 1974; Béress et al., 1975). Subsequently, a lot of data have been published and the research is progressing to improve the knowledge of the bioactive effects of these toxins (Mariottini et al., 2015). For example; studies on Dalazatide, which is a peptide derived from Cnidaria, showed that it could be effective to treat autoimmune diseases such as psoriasis (Liao et al., 2019; Pennington et al., 2015). Cnidarian toxins are known to be active on Na^+ and K^+ channels (Bosmans and Tytgat, 2007), Acid Sensing Ion Channels - ASICs (Diocot et al., 2003, 2007; Yaroslav et al., 2012), and therefore they are able to induce remarkable neurotoxic effects (Vasconcelos Carneiro et al., 2001). Such effects were supposed to be useful in the treatment of neurogenerative disorders such as Parkinson's disease, in which inflammatory processes have a critical role during early genesis (Chen et al., 2012). Given their ability to interact with the voltage-gated ion channels, peptides extracted from the sea anemones *Heteractis crispa* and *Bunodosoma cangicum*, might be involved in chronic pain treatment, having analgesic effects induced via TRPV1 modulation (Yaroslav et al., 2012; Zaharenko et al., 2011). Also, pseudopterosin A and E, produced by the sea whip *Pseudopterogorgia elisabethae*, showed analgesic effects *in vivo* with ED₅₀ value of 4 mg/kg i.p. and 14 mg/kg i.p., respectively (Mayer et al., 1998). Another example of possible utilization concerns the N-terminal side of *Cyanea capillata*, *Aurelia aurita* and *Hydra magnipapillata* toxins, having α -helices and bacterial toxins structural analogues; they were shown to induce toxic effects to insect cells (Brinkman and Burnell, 2009; Brinkman et al., 2014) and can provide a basis for further insecticidal application studies (Yu et al., 2005). These, and also other aspects, as reported in some reviews (Rocha et al., 2011; Mariottini and Grice, 2016) make cnidarians very interesting for the possible utilization of substances or venoms that can be extracted from some anatomical parts (tentacles, oral arms) or from the whole body. Therefore, notwithstanding the utilization of cnidarian extracts at present is still scarce, a number of data on biological activity of compounds and extracts of jellyfish exist.

It is known that cnidarian venoms are stored into nematocysts, namely the specialized intracellular capsules (located into cnidocytes) which are the 'reservoirs' of toxic substances and act as stinging organelles; Weill (1930) distinguished three main groups of nematocysts, according to their morphology and internal structure. *Astomocnidae* are characterized by closed filament at the apical end, *stomocnidae* possess an open filament, while *spirocysts*, occurring only in anthozoans, possess a filament devoid of spines and shaft. Subsequently, Mariscal (1974) provided a comprehensive explanation and description of all known different types of cnidarian nematocysts. These structures have important ecological implications, because cnidarians developed stinging mechanisms and toxic substances in order to catch prey and to feed, or as a defense mechanism in the natural environment.

Venom injection is known to occur after a mechanical or chemical stimulation of the nematocyst, which induce filament extrusion and the consequent stinging (Mariscal, 1974; Allavena et al., 1998).

The study of nematocyst morphology, function and the toxicity of their compounds is a basic pre-requisite for evaluating their potential in drug discovery. This study aims therefore to evaluate these basic aspects in two cnidarian species: *Vevelia velella* of which cytotoxicity was never studied, and *Aurelia aurita* which in this field was traditionally scarcely considered (Helmholz et al., 2010; Lee et al., 2011).

Aurelia aurita (Linnaeus, 1758) is a scyphozoan medusa with a global distribution in temperate and cold-temperate waters; it is commonly found along the coasts of the Mediterranean and the Black Sea (Vaisseire, 1984; Kideys et al., 2000). They are often referred to as 'harmless jellyfish' because their nematocysts are able to induce only mild effects on humans (Kokelj et al., 1990; Yoffe and Baruchin, 2004). Nevertheless, extracts of *A. aurita* samples are known to contain proteolytic enzymes, which can irritate skin and eyes, and can interact with muscle membrane

of vertebrates, increasing its permeability to Na^+ ions (Kihara et al., 1988; Bayazit, 2004). *Aurelia aurita* extracts also show phospholipase A2 (PLA2) activity, a known contributor to animal venoms in terms of neurotoxicity, myotoxicity and haemolytic effects (Radwan et al., 2001).

Vevelia velella (Linnaeus, 1758) or 'by-the-wind sailor' is a colonial hydrozoan (Anthoathecata) which lives at the air-water interface and is distributed in warm and temperate waters (Purcell et al., 2012). *Vevelia velella* diet relies on other planktonic organisms such as copepods, euphausiid eggs, fish eggs, barnacle cyprids, appendicularians and others (Purcell et al., 2012, 2015). Predation is carried out throughout a peripheral ring of dactylozooids involved in prey immobilization and capture (Kirkpatrick and Pugh, 1984). Nematocyst clusters are located at the tentacle extremity, where they are used for feeding and protection duties for the colony.

2. Materials and Methods

2.1. Jellyfish specimens

Specimens of *V. velella* were collected along the coastline of Genova (Gulf of Genova, Ligurian Sea) during a typical and recurrent outbreak phenomenon (Fig. 1). Specimens of *A. aurita* were sampled in Güllük Bay, Southwest coast of Turkey, and in coastal waters of the Ligurian Sea in front of Genova. In Güllük Bay *A. aurita* ($n = 38$), having bell diameter size 10–21 cm, were collected by hand net from a boat, maintained in seawater and transported to Muğla Sitki Koçman University, Faculty of Fisheries, Marine Biology Laboratory. Specimens collected in the Ligurian Sea were maintained in seawater and immediately transferred to the laboratory of the University of Genova, where they were frozen until utilization.

2.2. Evaluation of nematocyst morphology

Marginal bell and oral arm parts of *A. aurita* specimens were cut and stored at -18°C . Samples were thawed in the refrigerator at $+4^\circ\text{C}$ for 24 h and shaken at 1–2 h intervals to isolate nematocysts. Then, the samples were mixed in a homogenizer for 5 min to remove the cells from

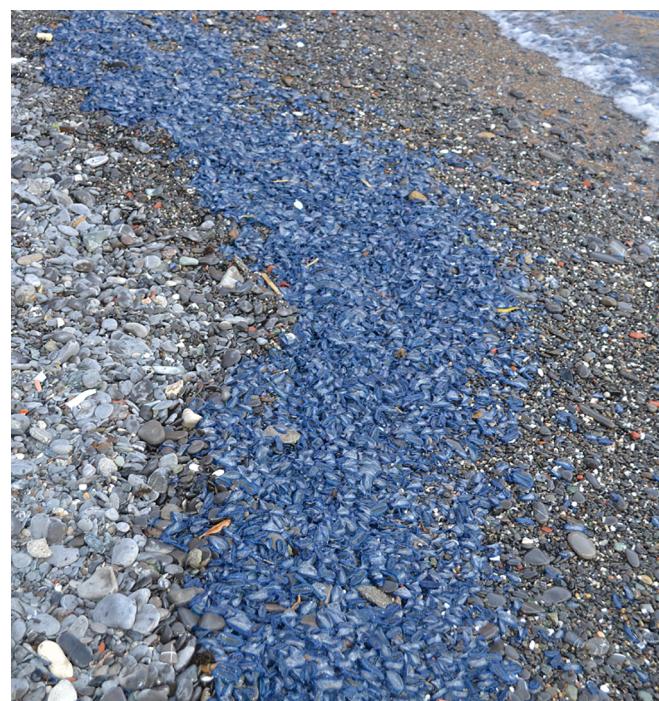


Fig. 1. Outbreak of *V. velella* and stranding along the Ligurian coast (Ligurian Sea). Photograph by G. Bonello.

the tissues and centrifuged (5000 rpm) at +4 °C for 5 min. Supernatants were removed and the residues were observed and counted under the light microscope examined with immersion oil at 1000× magnification. Three subsamples of 1 ml each were taken from each sample and examined. Nematocysts were identified according to Calder (1971) and Östman (2000). In addition, the length and width measurements of undischarged nematocysts were performed by micrometric ocular. The relationship between the number of nematocyst types and the diameter of the bell was determined by using correlation coefficient.

Specimens of *V. velella* were put in a beaker and homogenized with a magnetic stirrer in order to disaggregate tissues. Subsequently, the sample was centrifuged in Percoll gradient, according to Marchini et al. (2004) as modified by Brinkman et al. (2015), in order to separate nematocysts from tissue debris and jellyfish cells. Nematocyst morphology, which is largely unknown, was evaluated according to Calder (1971) and Östman (2000) by using a IX70 Olympus inverted microscope provided with image analyzer. Nematocysts were counted by using an hemocytometer Thoma.

2.3. Protein determination and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of *Velella velella* crude extract

Protein concentrations were determined by the method of Lowry et al. (1951), a colorimetric biochemical assay that combines the reaction of copper ions in alkaline conditions with Folin-Ciocalteu reagent, resulting in an intense blue molecule called heteropolymolybdenum blue. Protein content was then evaluated with a spectrophotometer (Analytic Jena Spekol 1300) at 660 nm. The calibration curve for the instrument was built with Bovine Serum Albumin (Sigma-Aldrich, Milano, Italy), and the sample analysis was performed in triplicate.

In order to compare the protein pattern of the *V. velella* crude extract samples pre and post nematocyst sonication step, the extract obtained as described in section 2.3 were subjected at SDS-polyacrylamide gel electrophoresis analysis (Laemmli, 1970).

The homogenates, sonicated or not sonicated, were centrifuged at 20,000×g for 30 min at 4 °C to remove bigger fragments and cell debris. The supernatant was collected, and protein content was quantified by Lowry assay (Lowry et al., 1951). Equal amounts of volume of each sample was mixed with the sample loading buffer (60 mM/L Tris-HCl, pH 6.8, containing 25% glycerol, 2% SDS, 0.1% bromophenol blue and 1% beta-mercaptoethanol) at 4:1 (v/v), heated at 100 °C for 5 min, and was loaded onto 12% polyacrylamide gel. The electrophoresis was carried out for about 1 h at a constant amperage of 45 mA on ice. After electrophoresis, the gel was fixed in 40% (v/v) ethanol, 10% (v/v) acetic acid for 60 min, washed twice in distilled water and finally stained with 0.1% (w/v) Coomassie blue R-250 in 45% (v/v) methanol and 10% (v/v) acetic acid overnight.

2.4. Preparation of *Velella velella* extracts for cytotoxicity tests

Frozen *V. velella* specimens were thawed and put in beakers placed in a magnetic stirrer without adding water, so that only jellyfish tissues and in-body water occurred in the extract. Stirring was prolonged for 24 h in order to disaggregate tissues; this procedure caused the release of a great number of intact nematocysts. Subsequently, nematocysts were counted by hemocytometer Thoma and the counting was taken as the main parameter to quantify venom amount. The crude extract so obtained was subsequently sonicated in ice bath at 30 s intervals for 90 times (totally 45 min sonication); with this method the discharge of around 80% of nematocysts was obtained. The sample was then centrifuged at 1250 rpm for 10 min and subsequently filtered through low protein binding 0.2 µm Millipore filters to remove all tissue debris.

2.5. Cell cultures

Established cell cultures of L929 mouse lung fibroblasts were

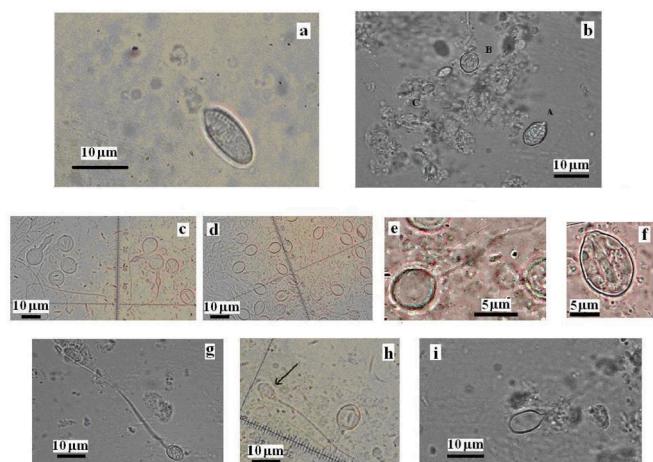


Fig. 2. Nematocysts in *A. aurita*. a: polyspiras (Southwest Aegean Sea); b: undischarged heterotrichous microbasic eurytele (A), discharged heterotrichous microbasic eurytele (B), a-isorrhiza (C) (Ligurian Sea); c: discharged euryteles (Marmara Sea); d: discharged euryteles (Southwest Aegean Sea); e: O-isorrhiza (Southwest Aegean Sea); f: birhopaloid (Southwest Aegean Sea); g: discharged A-isorrhiza (Ligurian Sea); h: discharged A-isorrhiza indicated by arrow (Southwest Aegean Sea); i: discharged polyspiras (Ligurian Sea). Photographs by Nurçin Killi and Sibel Cengiz (a, c-f, h) and Gian Luigi Mariottini (b, g, i).

purchased from the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (Brescia, Italy) and utilized to evaluate the cytotoxicity of crude extracts. Cells were maintained in DMEM medium supplemented with Foetal Calf Serum 5%, penicillin/streptomycin solution 1%, and l-glutamine 1% (EuroClone, Pero, MI, Italy), in humidified incubator at 37 °C temperature, according to standard culture methods.

2.6. Cytotoxicity tests

The number of nematocysts has been considered as the main parameter in the preparation of extracts. Seven doses of jellyfish crude extract, chosen on the basis of the number of nematocysts, were tested on cultured cells, starting from a dose of 150,000 nematocyst/ml and obtaining the subsequent doses after diluting the sample 1:1. The cytotoxicity of extracts was evaluated after 24 h treatment, by using the MTT assay method (Mosmann, 1983; Borenfreund et al., 1988); the readings were performed with a Zeiss Spekol spectrophotometer set at 570 nm wavelength. The IC₅₀ values were obtained by using the Trimmed Spearman Karber (TSK) method (Hamilton et al., 1977).

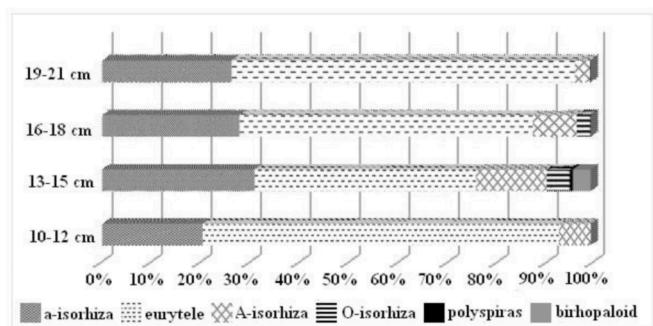


Fig. 3. Ratios of the nematocyst types in marginal bell samples of *A. aurita* according to the bell diameter.

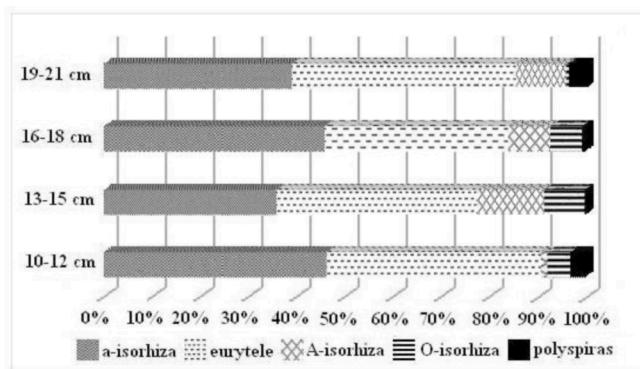


Fig. 4. Ratios of the nematocyst types in oral arm samples of *A. aurita* according to the bell diameter.

3. Results

3.1. Nematocyst morphology of *Aurelia aurita*

Six nematocyst types (a-isorhiza, A-isorhiza, O-isorhiza, eurytele, polyps and birhopaloid) were recorded in *A. aurita* samples (Fig. 2, a-i). In oral arm samples only five nematocyst types were observed, lacking birhopaloid. The most common nematocyst types were a-isorhizas and euryteles. O-isorhizas, birhopaloids and polyps were scarcely found and in a small number of individuals. Also, a correlation between total numbers of nematocyst types and bell diameters was not

Table 1
Minimum, maximum and mean length and width values of the nematocyst types observed in *A. aurita* specimens.

Nematocyst type	Min-max width (μm)	Min-max length (μm)	Mean length (±S.D) (μm)	Mean width (±S.D) (μm)
a-isorhiza	2–3	3–4	3.5 (±0.52)	2.5 (±0.52)
A-isorhiza	2–4	5–9	6.3 (±1.25)	3.3 (±0.82)
eurytele (small)	4–7	5–10	8.8 (±1.68)	5.8 (±1.03)
eurytele (med)	8–10	11–13	12.1 (±0.73)	9 (±0.81)
O-isorhiza	4–6	4–6	4.9 (±0.73)	4.9 (±0.73)
birhopaloid	9–10	11–13	11.5 (±1.35)	9.3 (±0.48)
polyps	2–5	6–13	8.5 (±2.46)	3.5 (±1.08)

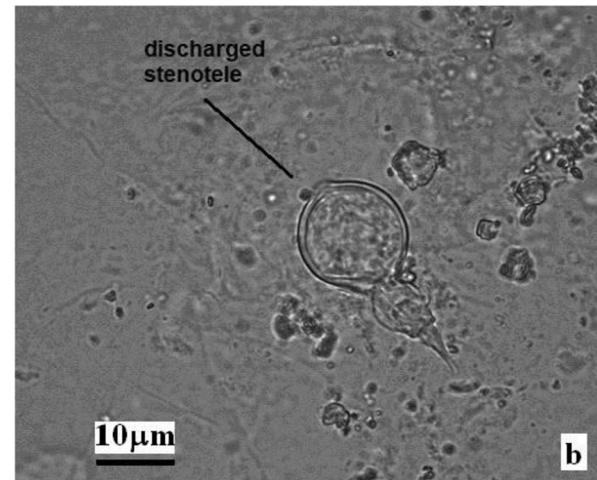
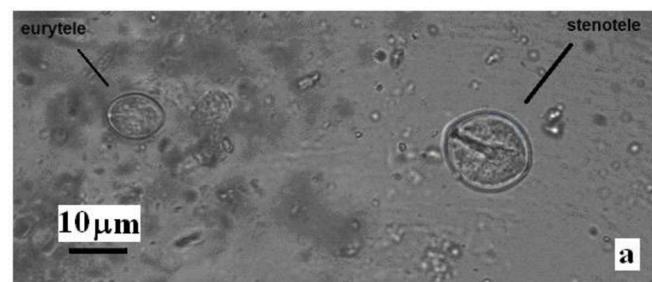


Fig. 5. Eurytele and stenotele nematocysts of *V. velella* from the Ligurian Sea (a); discharged stenotele in *V. velella* from the Ligurian Sea (b).

found (Figs. 3 and 4).

Euryteles in margin of the bell were the dominant nematocyst type in 10–21 cm bell diameter group. In oral arms, a-isorhizas were observed in high numbers in 10–12 cm and 16–18 cm groups, while euryteles were dominant in 13–15 cm and 19–21 cm groups.

Sizes of the nematocysts by each type in *A. aurita* are shown in Table 1. Euryteles showed two different sizes (small and medium) (Fig. 2, b-d). Some differences in shape of polyps of *A. aurita* between the Ligurian Sea and the Southwest Aegean Sea were determined. Polyps of *A. aurita* in the Ligurian Sea were elliptical shaped while that of the Southwest Aegean Sea were drop shaped, but length of polyps in both sampling areas are comparable (Fig. 2, a, i).

The numbers of nematocysts per specimen in *A. aurita*, distinguished according to the type, resulted on average $61.5 \pm 10.6 \times 10^4$ for A-isorhiza, $28.5 \pm 0.7 \times 10^4$ for heterotrichous microbasic eurytele, and $39.0 \pm 1.4 \times 10^4$ for polyps.

3.2. Nematocyst morphology of *Velella velella*

Only eurytele and stenotele nematocysts were observed in *V. velella* from the Ligurian Sea (Fig. 5). Stenoteles were found in higher numbers than euryteles. Also, the size of euryteles was 8–10 μm length and 6–8 μm width, while length and width values of stenoteles were 15–18 μm and 10–14 μm, respectively (Fig. 5). The numbers of nematocysts per specimen in *V. velella* resulted on average $34.2 \pm 0.7 \times 10^4$. *Velella velella* was not observed in Turkish coasts yet. Therefore, this species can not be compared in the two regions.

3.3. *Velella velella* crude extract: quantitative and qualitative analysis of protein content and cytotoxicity evaluation

Total protein determination in *V. velella* crude extracts was carried out before and after sonication in order to evaluate the difference

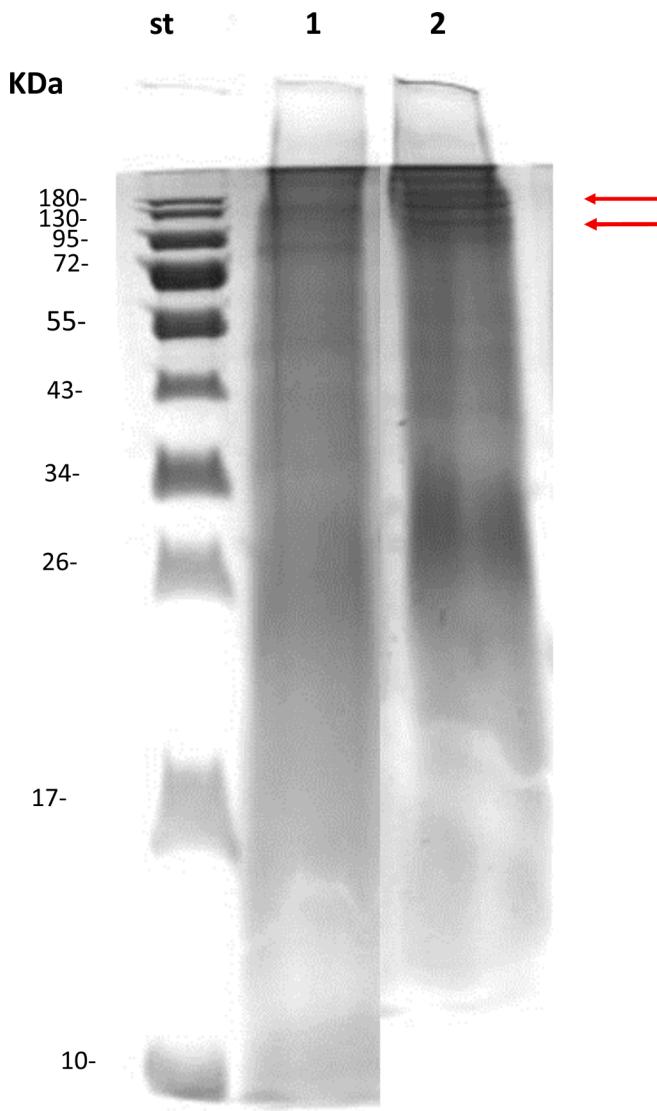


Fig. 6. SDS-PAGE analysis of *V. velella* crude extract before and after sonication. Equal volume of unsonicated (lane 1) or sonicated (lane 2) crude extract of *V. velella* were analyzed in a 12% polyacrylamide gel and stained with Coomassie Blue, as described in “Materials and Methods” section. st: Standard molecular markers. Red arrows show the protein bands exclusively detected in the sonicated sample.

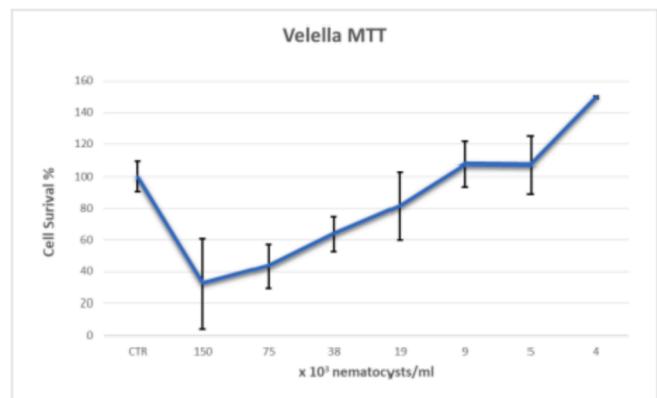


Fig. 7. Cytotoxicity of *V. velella* crude extract to cultured L929 cells. Cell survival rates at different tested doses.

between a sample with intact nematocysts and a sample with broken nematocysts; the unsonicated sample showed 0.18 ± 0.02 mg/L proteins, while the sonicated sample resulted in 0.24 ± 0.003 mg/L proteins; this result clearly indicates the contribution of venom leaked out from broken nematocysts.

The comparison of the SDS-PAGE pattern of these two samples, as shown in Fig. 6, reveals that in the unsonicated sample two main bands, located at 130 kDa and between 95 and 72 kDa, respectively, are visible while in the sonicated extract the number of bands rises to 4. In fact, in this sample in addition, two new protein bands located between 95 and 130 kDa and at 180 kDa, respectively (Fig. 6, red arrows), are present. These last proteins, exclusively represented in the sonicated extracts, could be released during the nematocysts lysis and could be the main proteins stored into the nematocysts of this animal.

The crude extract from *V. velella* caused remarkable decrease (40.5%) of cell survival at the highest dose (150,000 nem/ml). The treatment with the other doses showed a progressive increase of cell survival up to the dose 19,000 nem/ml. The lowest doses showed unexpected behavior, causing increase of cell proliferation or increase of the metabolic activity of L929 cells (Fig. 7). After treatment of L929 cells with crude venom of *V. velella* the IC₅₀ value, obtained with Trimmed Spearman Karber method (Hamilton et al. 1977), resulted 90.99 nematocysts/ml (N/ml) with confidence limits 57.13 N/ml (lower) and 144.92 N/ml (upper).

4. Discussion

Aurelia aurita species which are distributed in different regions of the world show different effects on humans. For example, it was reported that *A. aurita* in the Red Sea are more toxic than in the Chesapeake Bay (Radwan et al., 2001). Likewise, this species has strong toxicity in Mexico Gulf, Australia, Florida coasts and Israel (Radwan et al., 2001; Burnett et al., 1988; Benmeir et al., 1990). Also, it was determined that nematocyst morphology of this species was different in the two region of Turkey (Cengiz, 2019). Three individuals of *A. aurita* were sampled from izmit Bay in The Marmara Sea. It was observed that length and width values of the nematocysts of *A. aurita* in the Marmara Sea were higher than that in the Aegean Sea. It was found euryteles of the species in the two regions were different in shapes and sizes. Euryteles were round shaped in *A. aurita* of the Marmara Sea while they were drop shaped in the Southwest Aegean Sea (Fig. 2; c, d). The number of nematocysts (N) in the Marmara Sea (413 N/ml) was higher than that of the Southwest Aegean Sea (169 N/ml). So, it was thought that different salinities, food types and amounts might affect nematocyst numbers and morphologies.

Östman and Hydman (1997) recorded six nematocyst types (a-isorrhiza, A-isorrhiza, eurytele, birhopaloid, polyspiras, haploneme) in *A. aurita*. Also, a-isorrhiza was the dominant nematocyst type in polyps, but eurytele and a-isorrhiza were the most observed types in the medusa. In our study, parallel to Östman and Hydman (1997), eurytele and a-isorrhizas were found in high numbers in all bell diameter groups of medusa.

Nicholas and Yong (2012) studied length and width values of a-isorrhizas and euryteles in *A. aurita*. Mean length of a-isorrhizas were 4.90 ± 0.18 μm , mean widths were 3.35 ± 0.11 μm . In this study, mean length and width values of a-isorrhizas were measured as 3.50 ± 0.52 μm and 2.50 ± 0.52 μm , respectively. Euryteles were bigger in our study than recorded in Nicholas and Yong (2012) (8.15 ± 0.60 μm and 6.65 ± 0.33 μm , respectively). Mean length and width of euryteles reached 12.10 ± 0.73 μm and 9.00 ± 0.81 μm , respectively. This shows that the nematocyst size of individuals of the same species in different regions may be different.

Studies on nematocyst types and morphologies of *V. velella* are very scarce. Russell (1939) first studied the cnidome of *V. velella*, and identified atrichous ($8.9 \times 4.5 \mu\text{m}$) and stenotele nematocysts. Schuchert (2010) found stenotele, microbasic eurytele and isorrhiza in adults and two nematocyst types (stenotele and eurytele) in newly released medusa

of *V. velella*; minimum and maximum length-width of stenoteles were $18\text{--}21 \times 14\text{--}16 \mu\text{m}$ and $13\text{--}15 \times 10\text{--}11 \mu\text{m}$. Further observations showed patches of about 50 nematocysts along the external side of tentacular bulbs and irregular lines of nematocysts reaching the bell apex, while the external side of tentacular bulbs carry 15–20 nematocysts forming a line which extends to the bell apex (Schuchert, 2010). Recently (Manuele, 2019) reported the occurrence of stenotele and atrichous isorhiza nematocysts in colonies of *V. velella* having different sizes. Nematocysts were observed mainly in dactylozooids, gonozooids, and rim of the pneumatophore, while pneumatophore was completely devoid of stinging capsules. On the whole, a clear regionalization and a relationship between size of specimens and cnidome, with increasing of nematocyst size with specimen size were observed (Manuele, 2019).

Here we determined eurytele and stenotele nematocysts in *V. velella* from the Ligurian Sea. It was observed that shapes and sizes of the euryteles of *A. aurita* were similar both in Ligurian Sea and the Southwest Aegean Sea. However, euryteles of *A. aurita* from the Marmara Sea were different in shapes. Also, bell diameters and number of nematocyst types were not correlated in *A. aurita*.

Our results show that the bulk of protein content in *V. velella* is stored in nematocysts, as verified by the difference between the total protein amount in the unsonicated and sonicated samples. This result was confirmed by the comparison of the SDS-PAGE profile of the *V. velella* crude extract before and after the sonication. The presence of two new high molecular protein bands exclusively in the sonicated sample suggests a possible release of these molecules during the nematocysts destruction.

The cytotoxic properties of crude extracts from *V. velella* have been evaluated first in this research. We found that doses of 150,000 nematocysts/ml caused strong cytotoxicity to lung fibroblasts in culture. This result is quite similar to what is known for other jellyfish species, such as *Pelagia noctiluca* (Mariottini et al., 2002). Nevertheless, the crude extract at the lowest doses induced cell proliferation and/or an increase of metabolic activity. These data can be interesting in the perspective of acting on cell growth by modulating the amount of administered extract for applicative purposes.

5. Conclusion

Considering that in cnidarians the bulk of active compounds are contained into nematocysts (Rocha et al., 2011), many bioactive compounds are expected to be extracted from these organisms. This research which can be taken into consideration, could be a dowel for the basic knowledge for future applications in the field of drug and bioactive substances discovery of two species which are known to be not highly venomous.

Author's contributions

“conceptualization, N.K. and G.L.M.; methodology, G.B. and S.C.; validation, N.K. and G.L.M.; formal analysis, N.K., G.B., P.P., G.L.M., M.P., and S.C.; resources, P.P.; data curation, G.B. and S.C.; writing—original draft preparation, N.K., G.B., G.L.M., and M.P.; writing—review and editing, N.K. and G.L.M.; supervision, N.K.; funding acquisition, N.K.”

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Declaration of competing interest

The authors declare no conflict of interest.

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