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NEMATOCYSTS TYPES AND MORPHOLOGICAL FEATURES OF SOME SCYPHOZOA SPECIES IN THE SOUTHWEST TURKEY

Sibel Cengiz, Nurcin Killi*

Mugla Sitki Kocman University, Faculty of Fisheries, Department of Basic Sciences, 48000, Mugla, Turkey

ABSTRACT

In this study, it was aimed to determine the nematocyst types and morphologies and the relationship between the bell diameters and nematocyst numbers of some scyphozoa species in Muğla coasts, Turkey. Three scyphozoa species which are *Cotylorhiza tuberculata* (Macri, 1778), *Phyllorhiza punctata* Lendenfeld, 1884 and *Chrysaora hysoscella* (Linné, 1766) were sampled in Muğla coasts. Four different methods were experimented for nematocyst isolation but it was determined that the most effective method was the tissues were homogenized and then centrifuged. Four and seven nematocyst types were identified in *C. tuberculata* and *P. punctata*, respectively. Generally, euryteles were the most common nematocyst types in *P. punctata* and O-isorhizas were the dominant types in *C. tuberculata* samples. Five nematocyst types identified which were a-isorhiza, A-isorhiza, O-isorhiza, eurytele and polyspiras in *C. hysoscella*. In *C. tuberculata*, correlations between bell diameters and numbers of nematocyst types were found in A-isorhiza and O-isorhiza (r=0,95; r=0,39, respectively) in margins and in all nematocyst types except O-isorhiza in oral arms. In *P. punctata*, only correlation was found between the numbers of O-isorhiza and bell diameters (r= 0,53) in the margin. In oral arms, numbers of a-isorhiza and O-isorhiza were correlated with bell diameters (r=0,74, r=0,56, respectively).

KEYWORDS:
Isorhizas, polyspiras, birhopaloid, stenotele, eurytele.

INTRODUCTION

Cnidaria species have special cells organelles called nematocyst used for defense and capture prey [1]. Nematocyst consist of a protein capsule filled with stinging venom and a tightly wound thread [2]. When contact with nematocyst with mechanical or chemical stimulants, the thread penetrates into the tissue and injects the venom contents [3]. Nematocysts are found intensely in tentacles, oral arms and margins.

Cnidarian venoms cause serious health problems in Asia, Australia and the Mediterranean Sea [4, 5, 6]. In the Mediterranean Sea, jellyfish are not extremely toxic to humans (7, 8), but some species such as *Physalia physalis*, *Carybdea marsupialis*, *Rhopilema nomadica* and *Pelagia noctiluca* are potentially dangerous for people [6].

There are thirteen scyphozoa species distributed in Turkey seas. Among these species, *Pelagia noctiluca* is a native of the Mediterranean and causes redness, edema, burning, blistering and excessive pain in humans (9, 10, 11, 12, 13). *R. nomadica*, one of the lesepsian species, forms aggregations on the Mediterranean coast of Turkey, blocking fishing nets and causing economic losses. In addition, it affects holidaymakers and causes hospital cases due to severe pain and wounds. (14, 15). *Chrysaora hysoscella* is one of the other effective stinging species distributed in the Mediterranean Sea. This species has severe burning feature with its long tentacles (16). *Rhizostoma pulmo* does not cause hospital cases, but it causes burning, redness, blistering and rashes (6). Scyphozoa species directly reduce fish populations by consuming fish eggs and larvae and indirectly feeding on zooplankton. They cause economic losses by damaging fishing and aquaculture activities by clogging fishing nets and accumulating in the cooling water pipes of power plants (17).

There are thirty types of nematocysts identified [18, 19, 20, 21, 22, 23, 24, 25, 26, 27]. Nematocyst types have different morphology and different venom contents. Also, different venoms have different effects on organisms such as paralytic, neurotoxic, cytotoxic, dermotoxic and haemolytic [6, 28, 29, 30].

Sutton and Burnett [31] identified ten different nematocyst types in *Chrysaora quinquecirrha*. In addition, nematocyst morphology of *Cyanea capillata* (32), *C. lamarckii* [27, 32], *Pelagia noctiluca* [33, 2, 34], *Rhopilema nomadica* (35), *Catostylus mosaicus* and *Phyllohiza punctata* [36] were studied. In Turkey, there are few studies on the morphology of nematocyst of some species which are *C. andromeda*, *C. hysoscella*, *A. aurita* and *C. tuberculata* [37, 16, 38]. Therefore, in this study, it was aimed to determine the nematocyst types and morphologies and the relationship between the bell diameters and nematocyst numbers of some scyphozoa species in Muğla coasts, Turkey. Nematocysts are mostly found in tentacles, mouth arms and margins. In addition, nematocysts rates in different body parts was also determined in this study. Besides, it was experimented different methods for the nematocyst isolation and the most effective method was stated.

MATERIALS AND METHODS

Three scyphozoa species which are *Cotylorhiza tuberculata* (Macri, 1778), *Phyllorhiza punctata* Lendenfeld, 1884 and *Chrysaora hysoscella* (Linné, 1766) were sampled in Muğla coasts. Thirty-five *C. tuberculata* were sampled from Gökova Bay in September 2017 and fourty seven *P. punctata* were taken from Sülüngür Bay, Köyceğiz Dalyan Lagoon System in September and October 2017 (Fig. 1). Only one individual *Chrysaora hysoscella* was found in August in Güllük Bay. Samples were collected by a hand net on the boat and conveyed to Muğla Sıtkı Koçman University, Faculty of Fisheries. Bell diameters of the samples were measured and then parts of both margins and oral arms were taken into 5 ml sample vessels. These samples were stored in -18°C.

Four different methods were experimented for nematocyst isolation [39, 36, 40, 5]. These methods were described below:

1) Homogenizing and the centrifuging the tissues
2) Homogenizing and filtering through 100 µm mesh size sieve
3) Crushing in morter and filtering through 100 µm mesh size sieve
4) Squashing directly a small part of tissue on slide coverslip

It was determined that the most effective method was the first one that the tissues were homogenized and then centrifuged. In the other three methods, it was seen that the nematocysts did not completely separate from the tissue. When the samples used, they were defreezed in the refrigerator at +4°C and were shaken one or two hours' intervals. The samples were then mixed in the homogenizer to smash the tissues. Then the samples centrifuged for five minutes at 5000 rpm at +4°C. Supernatants were removed and residues were observed under the light microscope and photographed. One millilitre of subsamples was counted with three replicates. Identification of nematocyst types was done according to Calder [41] and Östman [42]. Length and width of undischarged nematocysts were measured with micrometric ocular. Euryteles were identified according to their sizes as small (S), medium (M) and large (L). The relationship between the number of nematocyst types and the diameter of the bell was determined using the correlation coefficient.

RESULTS

Thirty-five *C. tuberculata* and forty-seven *P. punctata* individuals were sampled. Number of individuals sampled different size groups were shown in Fig. 2. Nematocyst ratios of margins and oral arms of each individual were determined. When the total number of nematocysts in millilitres were examined, it was found that there was no significant relationship between bell diameters and nematocyst numbers. Thus, the total nematocyst numbers in ml of the same species in the same bell diameter vary. In general, it was seen that the total number of nematocysts in the oral arm samples was higher than the margin samples.

In the margins of *C. tuberculata*, the dominant nematocyst type was O-isorhiza in the all bell diameter groups. The numbers of A-isorhizas and O-isorhizas were correlated with the bell diameters of the samples. The percentages of numbers of the nematocyst types according to bell diameters were shown in Fig. 3.

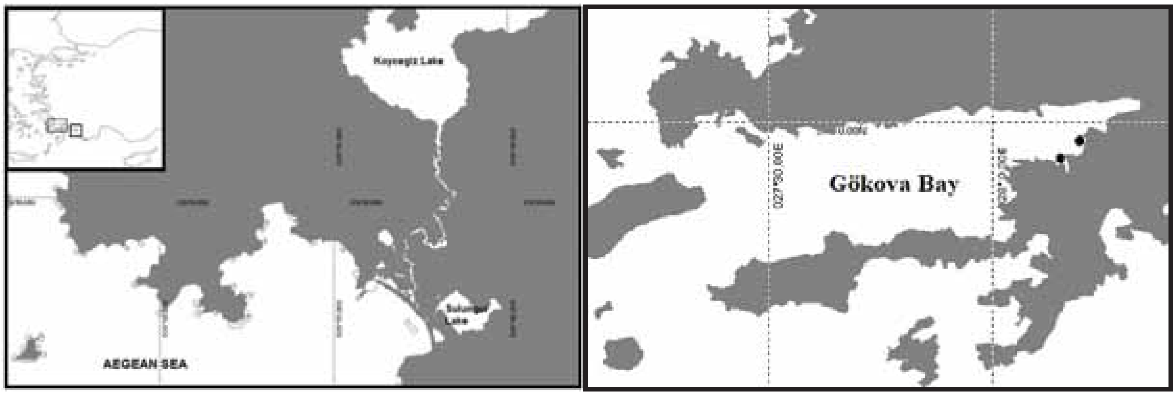

FIGURE 1
Jellyfish sampling areas (*P. punctata* in Sülüngür Lake and *C. tuberculata* in Gökova Bay).

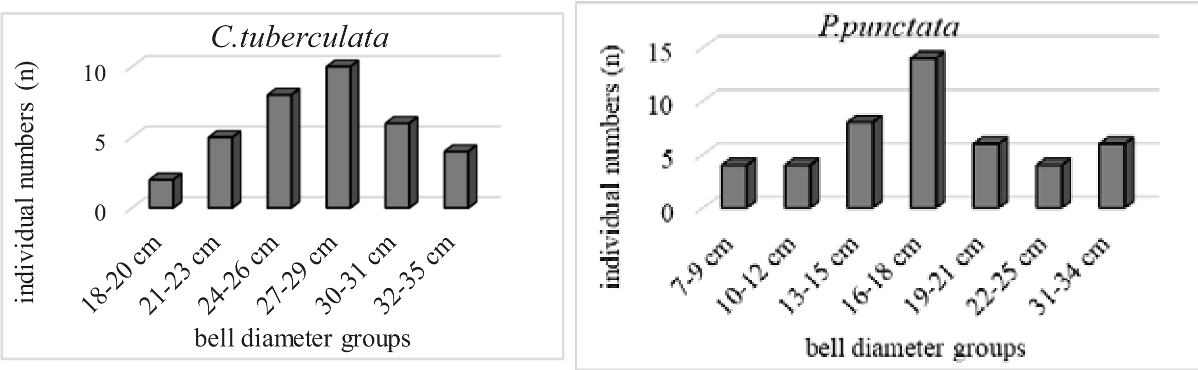

FIGURE 2
Numbers of scyphozoa samples according to the bell diameter groups.

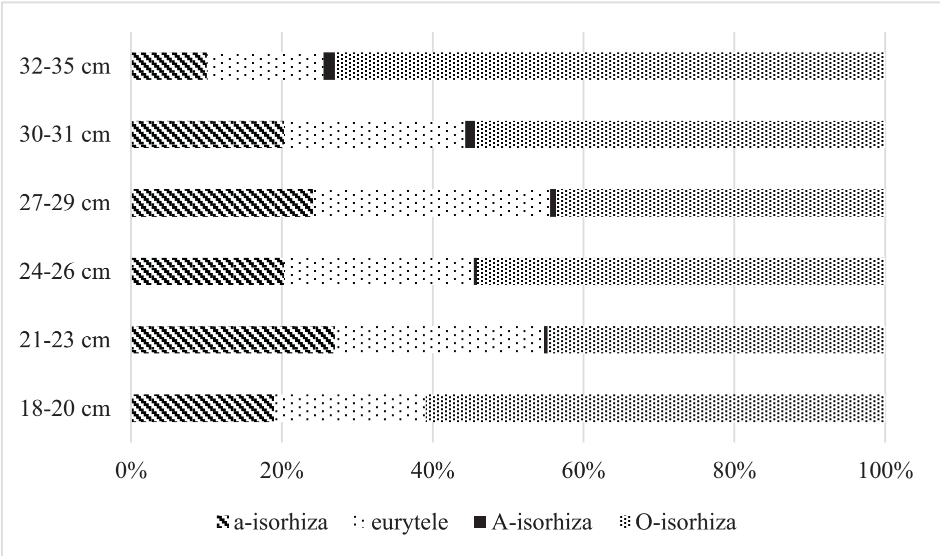

FIGURE 3
The percentages of nematocyst types of the margin samples in *C. tuberculata*.

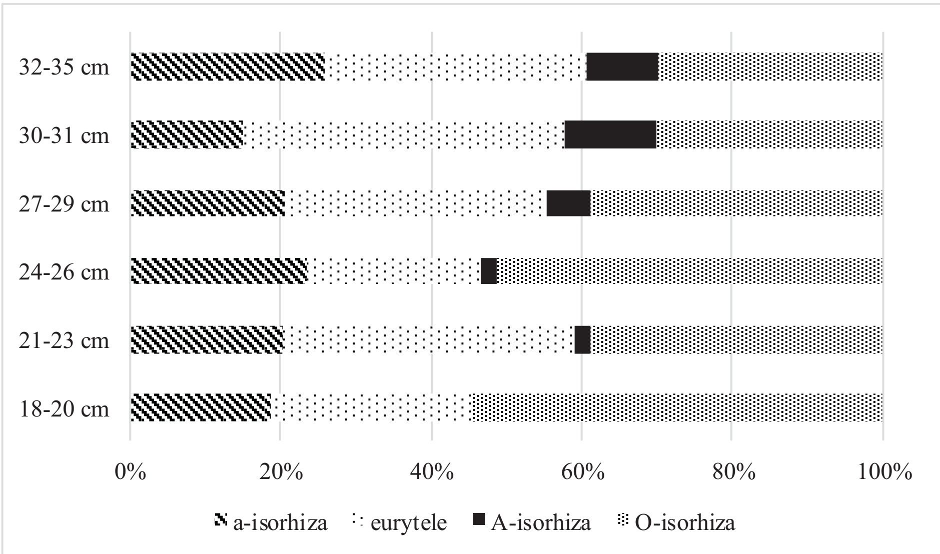

FIGURE 4
The percentages of nematocyst types of the oral arms in *C. tuberculata*.

In the oral arms, the dominant nematocyst type was eurytele in 30-35 cm bell diameter groups while O-isorhiza was the most common type in other bell diameter groups. It was found correlation between numbers of A-isorhizas (r=0,92), euryteles (r=0,57) and a-isorhizas (r=0,44) and bell diameters. But, A-isorhiza was not observed in the margin and oral arm samples of 18-20 cm bell diameter group (Fig. 4).

In the margin samples of *P. punctata*, the percentages and the most common nematocyst types according to bell diameter groups were shown in Fig. 5. Euryteles were the dominant nematocyst type in the margins of all the bell diameters except 31-34 cm group which was include commonly O-isorhizas. Only correlation was found between the numbers of O-isorhizas and bell diameters (r=0,53).

In the oral arms, O-isorhiza has the dominant nematocyst type in 31-34 cm bell diameter groups while eurytele was the most common type in other bell diameter groups. Polyspiras were observed in low number of individuals with few numbers (Fig. 6). Numbers of a-isorhiza and O-isorhiza were correlated with bell diameters (r=0,74, r=0,56, respectively).

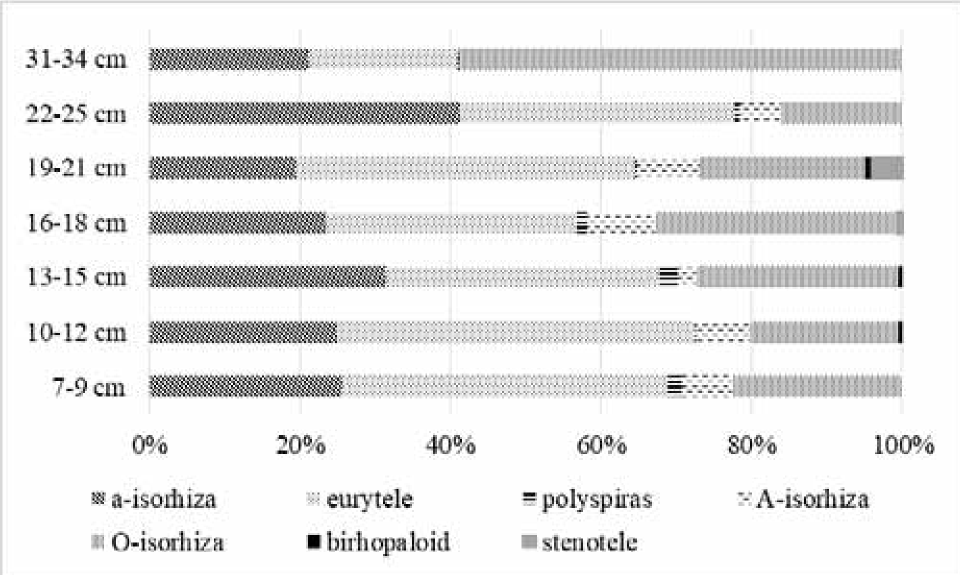

FIGURE 5
The percentages of nematocyst types of the margin samples in *P. punctata*.

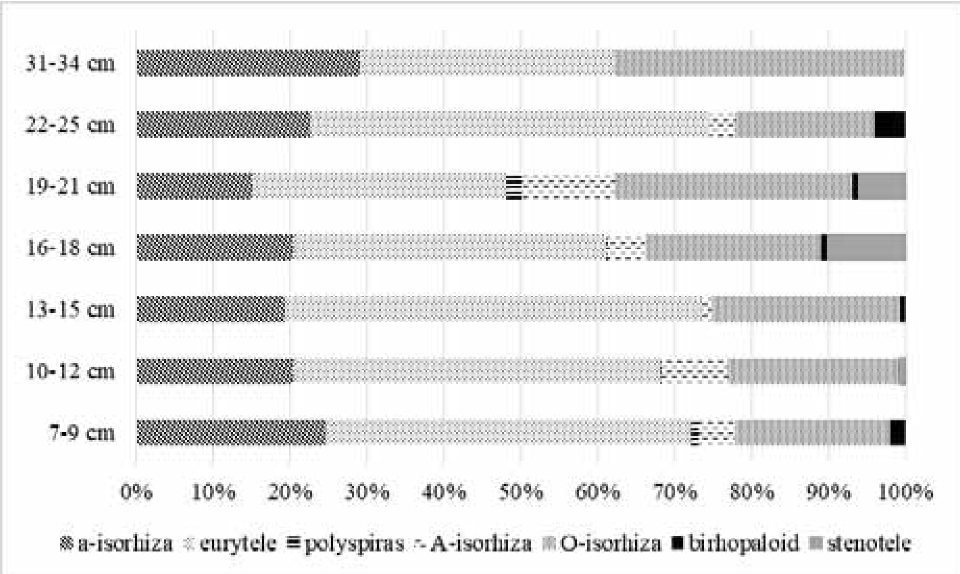

FIGURE 6
The percentages of nematocyst types of the oral arms in *P. punctata*.

TABLE 1
Length-width ranges and mean length-width values of the nematocyst types in *C. tuberculata* and *P. punctata*.

Species	Nematocyst types	Min-max width (µm)	Min-max length (µm)	Mean length (± S.D) (µm)	Mean width (± S.D) (µm)
Cotylorhiza tuberculata	a- isorhiza	2 -3	3-4	3,5 (± 0,52)	2,5 (±0,52)
	A- isorhiza	3	5 -6	5,3 (± 0,48)	3 (±0)
	eurytele (S)	2 -8	5 -10	8,2 (± 1,76)	5,8 (±2,11)
	eurytele (M)	8 -9	11 -12	11,2 (± 0,42)	8,4 (±0,51)
	O-isorhiza	2 -5	2 -5	3,6 (±1,07)	3,6 (±1,07)
Phyllorhiza punctata	a- isorhiza	2 -3	3 -4	3,5 (± 0,52)	2,5 (±0,52)
	A- isorhiza	2- 4	5 -6	5,3 (± 0,48)	3 (±0)
	eurytele (S)	4 -5	5- 7	6,4 (± 0,84)	4,9 (±0,56)
	eurytele (M)	6- 8	8 -10	9,6 (± 0,51)	6,6 (±0,51)
	eurytele (L)	9 -13	12- 20	14,4 (± 1,76)	11,05 (±1,28)
	stenotele	10-12	13-15	14,3 (±1,15)	11 (±1)
	O-isorhiza	3 -7	3 -7	5,1 (± 1,52)	5,1 (±1,52)
	polyspiras	2 -4	5 -7	5,7 (± 0,82)	2,7 (±0,82)
	birhopaloid	10 -15	12 -18	15,41 (± 1,83)	11,5 (±1,31)

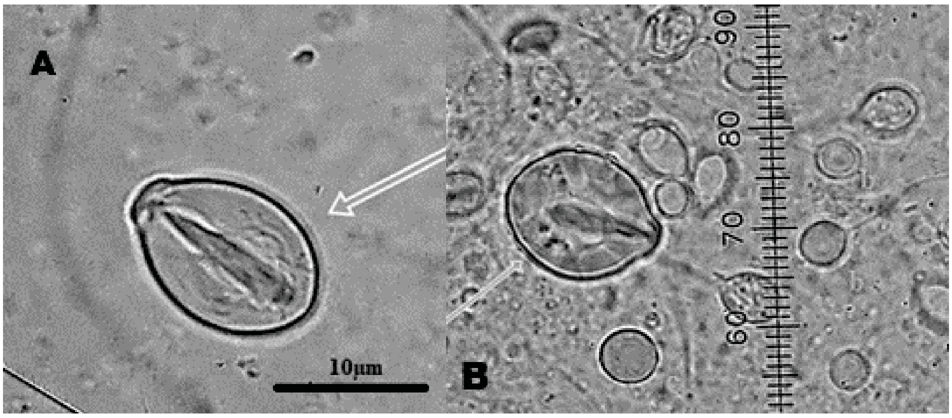

FIGURE 7
(A) Stenotele in *P. punctata*. (B) Undischarged birhopaloid (a), discharged O-isorhiza (b) and undicharged O-isorhiza (c) in *P. punctata*.

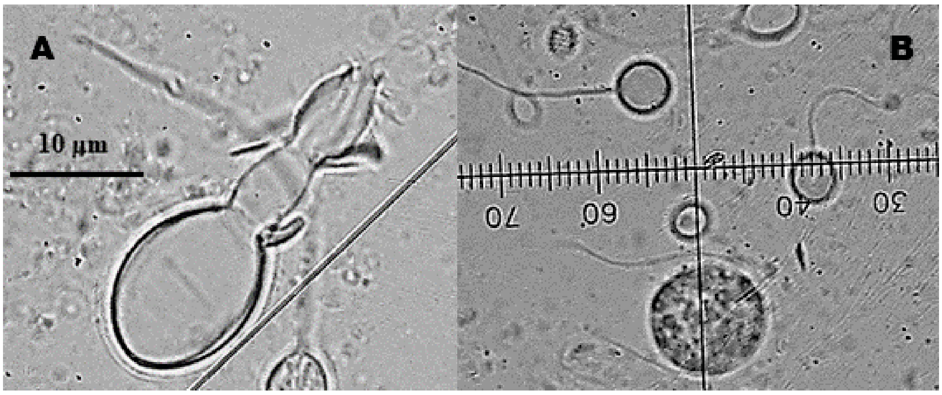

FIGURE 8
(A) Discharged birhopaloids in *P. punctata*. (B) Undischarged a-isorhiza (a), discharged O-isorhiza (b) and zooxanthella (c) in *C. tuberculata*.

It was observed that mean length and width values of a-isorhizas and A-isorhizas were not changed in both the species. Euryteles were found in different two sizes in *C. tuberculata* and three sizes in *P. punctata*. Sizes of O-isorhizas were bigger in *P. punctata* than that of *C. tuberculata* (Table 1).

Stenoteles, polyspiras and birhopaloid types were found only in *P. punctata*. Maximum length of birhopaloids was 18 µm and this type is distinguished from stenoteles by pads at the edge of capsule. Polyspiras which is oviform shaped was found in *Aurelia aurita* [43, 44]. Calder [43] recorded that length-width values of polyspiras were 8,8-12,9 µm and 4,4-5,9 µm in *A. aurita*. Length-width rates of *A. aurita* were measured as 6-13 µm and 2-5 µm [44]. In this study, length-width rates of polyspiras were 5-7 µm and 2-4 µm, respectively in *P. punctata*. Large euryteles were found only in *P. punctata* samples and mean length and width rates were 14,4±1,76 µm and 11,05±1,28 µm, respectively.

Only one specimen of *C. hysoscella* was sampled in Güllük Bay. Five nematocyst types identified which were a-isorhiza, A-isorhiza, O-isorhiza, eurytele and polyspiras in *C. hysoscella*. Maximum length-width rate of euryteles was 10-6 µm. Undischarged O-isorhizas had up to 20 µm length and width. Whereas maximum length and width rates of *C. tuberculata* and *P. punctata* were 7-5 µm. Thread length of discharged O-isorhizas was more than 300 µm. Mean length and width of polyspiras were measured as 8-3 µm, respectively. Also, mean length and width of A-isorhiza were 6-4,5 µm, respectively. In *C. hysoscella*, the most common nematocyst type was eurytele in margin and was O-isorhiza in tentacles.

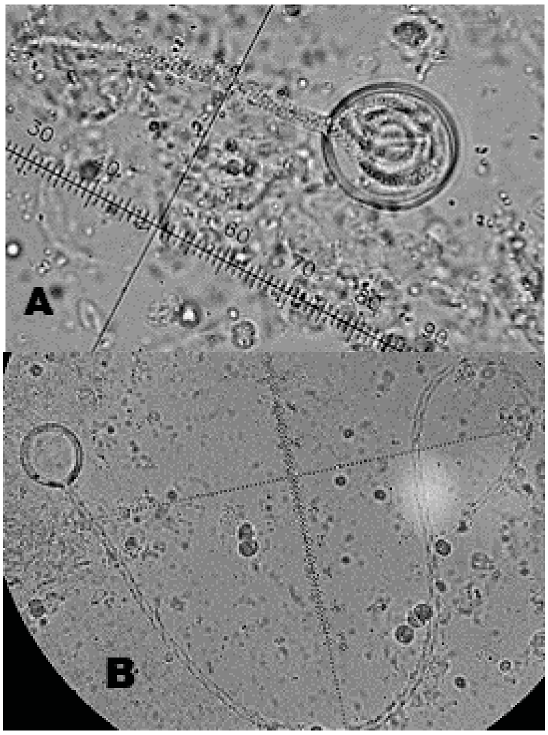

FIGURE 9
O-isorhiza that is being discharged (A) and discharged (B) in *C. hysoscella*

DISCUSSION AND CONCLUSION

Four, seven and five nematocyst types were identified in *C. tuberculata*, *P. punctata* and *C. hysoscella*, respectively. Generally, euryteles were the most common nematocyst types in *P. punctata* and O-isorhizas were the dominant types in *C. tuberculata* samples. In *C. hysoscella*, eurytele and O- isorhiza were the most common types in margin and tentacles, respectively. It was determined that numbers of different nematocyst types have changed even in individuals with the same diameter(Figure 9). Differences on nematocyst numbers may be caused difficulty of isolating nematocysts from the tissues. On the other hand, *P. punctata* and *C. tuberculata* are in symbiotic relationship with zooxanthellae. So, density of the zooxanthellae makes the isolation and counting of nematocysts difficult. Zooxanthellae were observed more intensively in *C. tuberculata* than *P. punctata*.

Bell diameter groups of *C. tuberculata* were 18-20 cm, 21-23 cm, 24-26 cm, 27-29 cm, 30-31 cm and 32-35 cm. Four nematocyst types which are a-isorhiza, A-isorhiza, O-isorhiza and eurytele, were identified both in oral arms and bell margins (Figure 8). In margins, the dominant nematocyst type was O-isorhiza. Also, correlations between bell diameters with A-isorhizas and O-isorhizas were observed (r=0,95; r=0,39, respectively). In oral arms, O-isorhizas and euryteles were the dominant nematocyst types. Correlations between bell diameters and nematocyst ratios were found in all nematocyst types except O-isorhizas in oral arms.

Seven nematocyst types were determined in *P. punctata* samples (eurytele, stenotele, a-isorhiza, polyspiras, A-isorhiza, O-isorhiza and birhopaloid). Euryteles in margin and oral arms were the most common nematocysts in this species. Birhopaloids were more in oral arms than in margins. Polyspiras were determined with low numbers in a small number of individuals. It was observed that, as the bell diameter increased, the number of nematocysts increased.

The biggest nematocyst type was birhopaloid and mean length-width values of it were 16,6µm±1,86 and 13,1 µm±1,73 in *P. punctata* (Figures 7,8). It was found that widths and lengths of a-isorhizas did not change in the two species. Length of A-isorhizas was up to 6 µm in the two species. Sizes of O-isorhizas were different in the *P. punctata* and *C. tuberculata*. Mean length values of O-isorhizas were 5,1±1,52 and 3,6±1,07, respectively.

Peach and Pitt [36] recorded 4 or 5 nematocyst types in *P. punctata* and they did not found all the nematocyst types in all individuals. Lengths and widths of a-isorhizas and euryteles were higher than in margins. Only relationship was found between bell diameters and a-isorhiza lengths. In this study, it was not found significant relationship between bell diameters and nematocyst lengths. As the bell diameter increased, the number of O-isorhiza increased (r=0,53).

Mean lengths and widths of *P. punctata* euryteles were 6,60±0,21 µm and 4,60±0,22 µm, respectively [45]. In our samples, euryteles were in three sizes and mean length-width values were 6,4±0,84 – 4,9±0,56 µm (small), 9,6±0,51 – 6,6±0,51 µm (medium) and 15,02±1,90 –

11,45±1,43 μm (large), respectively. Euryteles in the study of Nicholas and Yong [45] corresponded to small euryteles in this study. Also, euryteles of *C. hysoscella* were found as medium sized (10-6 μm). However, sizes of O-isorhizas were bigger than that of *C. tuberculata* and *P. punctata*. Maximum lengths of this type were 5 μm in *C. tuberculata*, 7 μm in *P. punctata* and 20 μm in *C. hysoscella*. (38) observed four nematocyst types (a-isorhiza, O-isorhiza, A- isorhiza and eurytele) in five individuals of *C. hysoscella* in March and April, 2012. a-isorhizas and O-isorhizas were the most frequently seen in the samples and O-isorhizas had up to 18 μm in length and width. Maximum length-width of euryteles were 9-7 μm in this species [38]. These results are similar to those in this study.

Östman [24], Östman and Hyman [27] identified birhopaloids in *C. tuberculata*. Also, Gülşahin [37] determined 35% euryteles, 24% birhopaloids and 41% a-isorhizas in this species. In this study, the most common nematocyst types were O-isorhizas (55,24%) and euryteles (24,37%) in *C. tuberculata*. It was not found birhopaloids in our samples. The reason for this is that there is a small number of birhopaloids and it cannot be isolated from the tissue.

Gülşahin [16] recorded two nematocyst types which were a-isorhiza and birhopaloid in *Cassiopea andromeda*. Also, a-isorhizas were the most common (61,11%) nematocyst types in this species. According to Calder [43] euryteles were abundant in *A. aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata*. Furthermore, shapes and sizes of nematocyst types varied both in different species and in polyps, scyphistomae and medusa of the same species [43, 46, 47]. Also, it was determined that euryteles of *A. aurita* which were sampled from different regions of Turkey were different shape. In the samples taken from the Gulf of Izmit, it was found that the euryteles were round shape and in the Muğla specimens as drop shaped [44, 48].

Calder [49] found isorhizas and euryteles in *Stomolophus meleagris* and classified the euryteles as small, medium and large. It was recorded that shapes and sizes of nematocyst types were different in developmental stages of this jellyfish (planula, ephyra, scyphistoma and medusa). In this study, we examined only the medusa of these two species. However, nematocyst numbers, shapes and sizes of different developmental stages of jellyfish should be investigated. This study, also will help to fill the scientific data and gaps in this subject.

The number of studies related to the nematocyst types and venom content of these species is few. Regional differences of nematocysts in *P. punctata*, *C. tuberculata* and other scyphozoans must be revealed. It must also be determined whether venom contents of same nematocyst types are regionally different.

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Conflict of Interest. The authors declare that there is no conflict of interests regarding the publication of this article.

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CORRESPONDING AUTHOR

Nurcin Killi
Mugla Sitki Kocman University,
Faculty of Fisheries,
Department of Basic Sciences,
48000 Mugla – Turkey

e-mail: nurcinkilli@mu.edu.tr