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## Pycnogonida (Arthropoda) from Northern Adriatic *Corallina officinalis* Linnaeus, 1758 belts

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### Abstract

This study examines Pycnogonida, sea spiders, collected from 54 *Corallina officinalis* belts in the vicinity of Pula and the Brijuni National Park. The areas include both exposed and sheltered localities as well as regions with various levels of human impact. Seven species were identified, namely *Achelia echinata*, *A. langi*, *Tanystylum conirostre*, *Anoplodactylus angulatus*, *A. pygmaeus*, *Trygaeus communis*, and *Callipallene tiberi*. As we used a quantifiable standard sample size of 5 cm<sup>2</sup>, we could perform a statistical analysis of species richness and abundance. The exposed low human impact sites showed a significantly higher amount of both, specimens and species than the sheltered high impact sites. *C. tiberi* and *A. echinata* showed a marked preference for exposed low impact sites while *T. conirostre* was equally distributed among the habitat subtypes.

**Keywords:** Sea spider; species distribution; exposition; human impact; statistical analysis.

### Introduction

The uppermost phytal community found in the mediolitoral and upper infralitoral rocky layers along the northern Adriatic coast is dominated by calcifying red algae, *Corallina officinalis*, Linnaeus, 1758. Due to its calcified thallus, it is more wave resistant than other algal communities and therefore forms the uppermost phytal layer at more or less exposed localities. *Corallina*'s fine ramifications provide a well-protected, quasi-hidden interstitial space inhabited by numerous microinvertebrates. In the northern Adriatic tide-dominated litoral zone, *Corallina* belts are the prevalent communities of calcifying red algae.

*Corallina* species play an important role in the marine ecosystem and are used in various indices to assess the ecological status of coastal waters in the Mediterranean, such as the cartography of littoral rocky-shore communities (CARLIT) (Ballesteros *et al.*, 2007, Nikolić *et al.*, 2013). Research around the world revealed the exceptional biodiversity of invertebrates living within the settlement of this alga (Kelaher *et al.*, 2001, Liuzzi & Gappa, 2008) which had yet to be confirmed also for the *Corallina* habitats in northern Adriatic. On the rocky coast of southern Istria, *C. officinalis* is frequently pres-

ent and can be found in isolated patches and in extensive turfs which are often recorded for this species (Hofmann *et al.*, 2011). As regarding its range of habitats, it can be found in exposed and sheltered bodies of water and on various artificial and natural substrates (Latham 2008). Differences between *Corallina* belts in sheltered and exposed localities in Norway are mentioned by Dommasnes (1968).

In the present study, we analyse pycnogonid communities associated with *C. officinalis* from various localities along the southern Istrian coast (Croatia). These have been sampled in the framework of a general inventory of *Corallina*-inhabiting invertebrates (Buršić *et al.*, 2019).

Pycnogonids live a cryptic life and are ubiquitous in all benthic marine environments. Pycnogonids collected from phytal communities have been studied at various localities in the Mediterranean. For example, Haro (1965, 1966, 1967) studied pycnogonids found in *Posidonia* as well as algal communities, and Arnaud (1987), and Arnaud & Bamber (1987) summarized their own and previous results in a general survey of the association of pycnogonids with the main types of marine habitats, with one of the main results being that *Halopteris scoparia* (Linnaeus) Sauvageau, 1904, a brown alga from the rocky upper infralittoral, carries the largest number of pycnogonid

species (see also Schüller 1989). Analyses of pycnogonid communities in the Mediterranean dealing with quantitative aspects are found, e.g., in Munilla (1991), Pérez-Ruzafa & Munilla (1992).

Regional studies of Istrian fauna including data on pycnogonids and their distribution in the upper layers of the benthic habitats are mostly from the area around Rovinj. Zavodnik (1968) and Krapp-Schickel & Krapp (1975) studied phytal communities on hard bottom, Schüller (1989) focused on *H. scoparia*. Moreover, Montoya Bravo *et al.*, (2006) studied a pycnogonid community under infralittoral stones at Cap Savudrija in the northern sector of the Istrian coast.

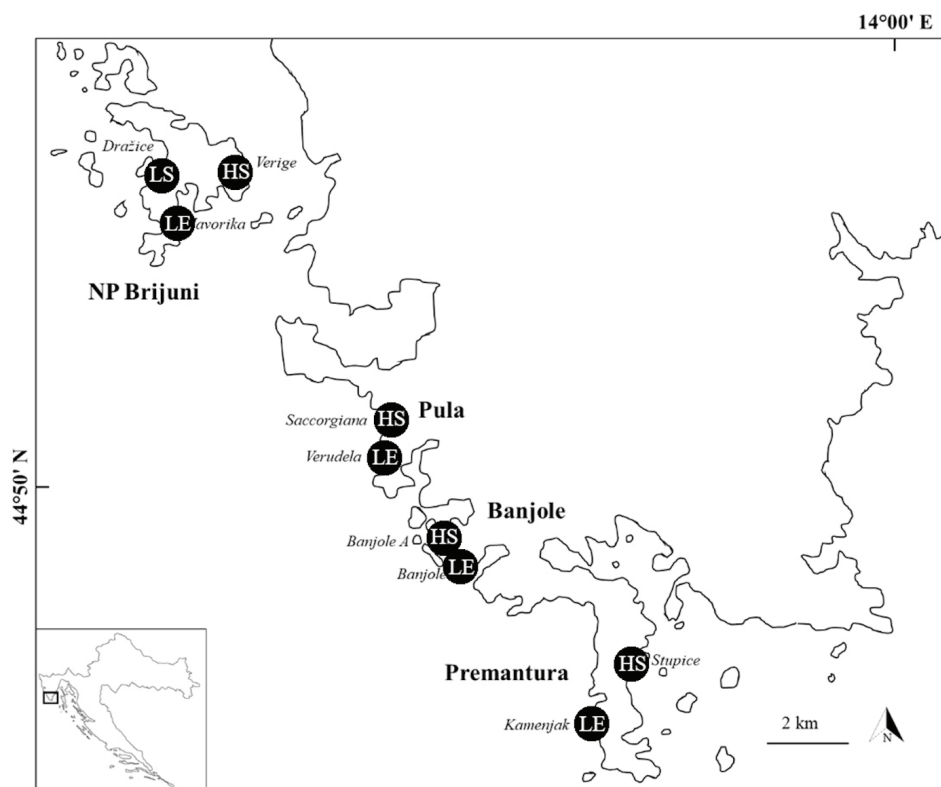
However, the *Corallina* belt has not been studied quantitatively in these analyses. Pycnogonid-*Corallina* associations have been studied at only a few localities around the Mediterranean, the Mersin Bay/Turkish Mediterranean coast (Kocak & Alan, 2013; Kocak, 2014), the Aegean Sea/Turkish coast (Arnaud 1976), and around Marseille (Arnaud & Bamber 1987). At the least, Arnaud (1987) provides a summary of which species have been found in which algal communities including also *Corallina* (see also Munilla & Soler-Membrives (2014) for a summary of more recent results on waters off the Iberian Peninsula).

The *Corallina* inventory that is currently being undertaken along the southern Istrian coast thus provided the opportunity to analyse pycnogonid-*Corallina* associations for the Adriatic. Since standardized *Corallina* sample sizes were used (25 cm<sup>2</sup>), in addition to species lists, we provide data on the absolute and relative abundance of pycnogonid species including statistical analyses.

## Materials and Methods

Samplings were done on 9 different locations off the southern Istrian peninsula and Veliki Brijun island in the Brijuni National Park between November 2017 and April 2018 when the density of algae was at its peak (Fig. 1) (Supplementary Table 1). The locations were chosen based on visual assessment of *Corallina* abundance and presence or absence of anthropogenic impact. At each site, two locations were selected in a way that one would be more impacted by human activity (either by the vicinity of small harbours, public beach, sewage discharge) and the other would be more distant from those kinds of activities or it would be in a marine protected area such as the Brijuni National Park and Kamenjak. To categorize a site exposed or sheltered, its exposure to winds and waves were taken into account, as well as its specific geographical location. Sampling sites situated in the inner part of bays and protected from wind and waves were considered sheltered, whereas sites on the outer parts of bays and convex coastal areas, where winds and wave action are common were considered exposed. In order to quantify the presence of anthropogenic impact, i.e., to assess the impact of land on coastal areas, the Land Uses Simplified Index (LUSI index) was calculated (Flo *et al.*, 2019), which resulted in lower values for low impact sites and higher values for high impact sites.

At all localities, sampling was done where the algal coverage was above 90% and where its distribution was continuous along the coastline. The width of the measured coastal continuous belt was about 50 cm. Coastline slope was always horizontal to minimize the differ-



**Fig. 1:** Sampling locations off the southern Istrian peninsula; L low human impact, H high human impact, E exposed, S sheltered.

ence between sampling locations and sampling was done when the algae were out of the water to minimize the loss of mobile invertebrates. Low impact sites were Verudela, Banjole, Kamenjak, Dražice, and Javorika and high impact sites were Saccorgiana, Banjole A, Stupice, and Verige.

Samples were taken during low tide when the algae was completely out of the water, stored in plastic containers, and fixed with a neutralized 4% seawater solution of formalin. On each location sampling was done quantitatively by scraping off two sets of three replicate quadrats 5x5 cm in size with the help of a hammer and chisel. Each set of replicates was at least 100 meters from the next, which resulted in a total of 54 samples analysed for this study representing 1,350 cm<sup>2</sup>. In the laboratory, samples were rinsed through a sieve with a 500 µm mesh size. Pycnogonids were sorted and counted under a stereomicroscope, preserved in 75% ethanol, and identified to the species level according to Dohrn (1881), Bouvier (1923), Stock (1952), Bamber (2010) and Lehmann *et al.*, (2014). The statistics were performed using software package Past, version 3.26b (Hammer *et al.*, 2001).

## Results

In total, seven pycnogonid species were found: *Achelia echinata* Hodge, 1864, *A. langi* (Dohrn, 1881), *Tanystylum conirostre* (Dohrn, 1881), *Anoplodactylus angulatus* (Dohrn, 1881), *A. pygmaeus* (Hodge, 1864), *Trygaeus communis* Dohrn, 1881 and *Callipallene tiberi* (Dohrn, 1881). Also, a couple of juvenile specimens could be identified only tentatively. The pycnogonid species com-

position and abundance of each sample locality are summarized in Supplementary Table 1. At most sample localities, one to three species were recorded, but in Banjole and Javorika, there were 4 and 5, respectively.

Table 1 shows that three species dominate the *Callipallene* habitat in the study area. By far the most common is *Callipallene tiberi*, accounting for ca. 58% of all specimens. In the second place is *Tanystylum conirostre* (ca. 18%), followed by *Achelia echinata* (ca. 9%). The other four species are represented by only a few specimens each. Six of the seven species were found in both, exposed and sheltered localities. *Trygaeus communis* was found only in exposed sites. About 10% of the studied pycnogonids were juveniles.

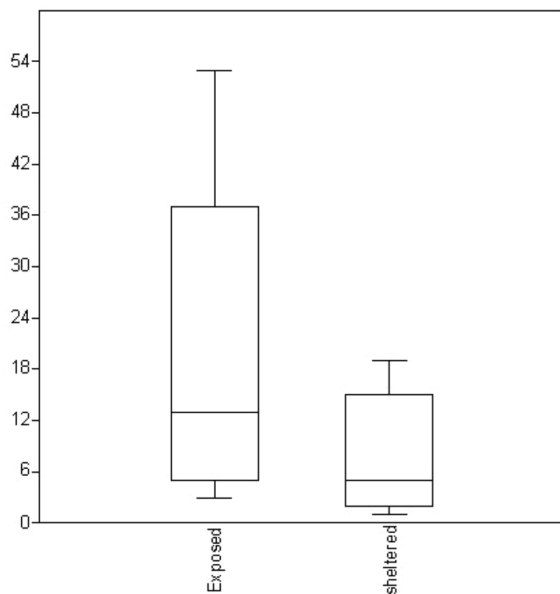
Table 1 and Figure 2 indicate that pycnogonids may be more abundant in the exposed than in the sheltered localities and that some species, e.g., *Callipallene tiberi*, may show an explicit preference for the exposed habitats, while *Tanystylum conirostre* might show no preference for any of the habitat subtypes.

Hence, to evaluate the distribution of Pycnogonida in relation to the exposition and human impact, a statistical analysis was done using strictly non-parametric statistics. For two independent samples, the Mann-Whitney-U-test is “best practice”, provided we have ranked data (Lamprecht, 1992).

One main problem with the sampled sites has to be faced: the parameters “human impact” and “exposition” are not independently distributed. The 21 samples with high human impact are all in sheltered localities, while within the 30 samples with low human impact, 24 come from sites in an exposed position and only 6 from a sheltered position, all of them from the same lo-

**Table 1.** Species list and number of specimens found for each species altogether and the different habitat subtypes.

Species [number of localities]	Number of specimens	Low impact & exposed = exposed [24]	Low impact & Sheltered [6]	High impact & sheltered [21]	Sheltered [27]
<i>Achelia echinata</i>	21	20		1	1
<i>Achelia cf. echinata</i>	1			1	1
<i>Achelia langi</i>	8	7		1	1
<i>Achelia cf. langi</i>	2	2			
<i>Achelia</i> sp., juvenile	7	6		1	1
<i>Tanystylum conirostre</i>	46	21	1	24	25
<i>Trygaeus communis</i>	2	2			
<i>Anoplodactylus angulatus</i>	4	3		1	1
<i>Anoplodactylus pygmaeus</i>	7	6	1		1
<i>Anoplodactylus cf. pygmaeus</i> , juvenile	5	4	1		1
<i>Callipallene tiberi</i>	124	95		29	29
<i>Callipallene cf. tiberi</i>	2	1		1	1
Number of specimens	229	167	3	59	62
Number of taxa	7	7	2	5	7



**Fig. 2:** Box plot of the number of pycnogonids found in exposed and sheltered localities.

cation (Dražice). This means that we can only discriminate between two parameter complexes (high impact and sheltered versus low impact and exposed). The Dražice samples are also poor in their content: only 3 pycnogonids in presumably two species have been found in them. Three samples were without pycnogonids. This does not allow any statistically relevant evaluation and means that the Dražice samples had to be omitted from the statistical evaluation. Banjole A1 samples were also omitted because no pycnogonids were found there as well. This has the additional advantage that both sample sizes are now more equal (24:21 instead of 30:24). Fortunately, the size of the smaller sample is high enough to calculate the U-values directly and it was not necessary to exclude them from tables.

With this reduced dataset the following differences could be found:

1. The exposed low impact sites show a significantly ( $p < 0.05$ ) higher number of specimens.
2. The exposed low impact sites show a highly significant ( $p < 0.001$ ) higher number of species in the single samples.

Only two species (*C. tiberi* and *T. conirostre*) have been collected regularly enough to allow a statistical evaluation with the U-test on species level.

3. *C. tiberi* is occurring in higher numbers at the exposed low impact sites, but the result is not significant at the 5% level.
4. In *T. conirostre* no statistically significant differences were found. The p-value (about 0.67) supports the hypothesis that no differences in specimen densities exist.

As we have a lot of zero values in single species evaluations, another evaluation was made using the Chi-squared test for “goodness-of-fit”. It shows if the ob-

served specimen numbers deviate from a theoretical normal distribution, but patchy occurrences might suggest non-existing significances. As Spelda (1996) has shown, only differences with 7 specimens or more in one category and none in the other can reach a significance level below 0.05, provided both samples are of equal size. If at least one specimen occurs in the other sample, the larger sample needs a minimum of 9 specimens. These differences in specimen numbers, shown in tab. 1 of Spelda (1996) could be used to decide which species have been collected in too low numbers to reach a significance level. Based on this criterion only three species (*A. echinata*, *C. tiberi*, and *T. conirostre*) had been collected in high enough numbers that a “goodness-of-fit” analysis could be performed.

In *A. echinata* the chi-squared test reaches a high significance level ( $p < 0.01$ ), supporting the hypothesis, that this species is associated with exposed low impact sites. A more intensive study might confirm this on rank data (U-Test) too.

In *C. tiberi* the chi-squared test reaches a very high significance level ( $p < 0.001$ ), confirming the trend suggested by the U-test. A more intensive study might confirm this on rank data (U-Test) too.

In contrast, *T. conirostre* shows no significant deviation from a normal distribution. This means that this species is equally distributed in both types of sites (exposed low impact and sheltered high impact). The result also suggests that patchy distribution of the specimens plays rather a minor role in the pooled data.

It is also worth mentioning that specimen numbers, in general, reach an even higher significance level than any single species, as they are on the one hand mainly influenced by the individuals of *C. tiberi*, and on the other hand, all species beside *T. conirostre* occur in higher numbers at the exposed low impact sites. Even if they do not reach significance levels themselves, they contribute to the overall significance of the specimens, as they do in the U-test.

## Discussion

With 229 specimens out of altogether 54 sampling localities corresponding to 1,350 cm<sup>2</sup> *Corallina* belt thoroughly searched for pycnogonids, pycnogonids seem to be well represented in this habitat type. Moreover, the pycnogonid community of Northern Adriatic *Corallina* belts is dominated by three species, *Callipallene tiberi*, *Tanystylum conirostre*, and *Achelia echinata*. All three of them had been recorded before for *Corallina* belts in the western Mediterranean and the Aegean Sea (Arnaud 1976, Arnaud & Bamber 1987), and are also identified as *Corallina* inhabitants by Arnaud (1987). They are thus common species inhabiting various phytal communities in the upper water layers including *Corallina*, but not exclusively in *Corallina*. Conversely, the second species of *Achelia* found in *Corallina* in addition to *A. echinata* in this study, *A. langi*, is less common in phytal communities and seems to be a rare guest in *Corallina* (Arnaud, 1987).

Its domain along the Istrian coast is the bottom side of infralittoral stones covered with bryozoans, sponges, and other sessile invertebrates, where this species is quite common (Montoya Bravo *et al.*, 2006). *Trygaeus communis*, the only species that has been found in our samples in exposed but not in sheltered sites, has been recorded for the Istrian coast in earlier studies (Krapp-Schickel & Krapp, 1975, Schüller 1989, Lehmann *et al.*, 2014) and is usually found in phytal communities. *Anoplodactylus angulatus* has been recorded several times in *Corallina* belts (Arnaud 1987), while *A. pygmaeus* has only once been noted as an explicit *Corallina* inhabitant (Arnaud 1976). As *Achelia echinata*, *Anoplodactylus angulatus*, and *Tanystylum conirostre*, the latter species is a common inhabitant of phytal communities feeding on organisms attached to them (for review of pycnogonid feeding ecology, see Dietz *et al.*, 2018) The species composition of the pycnogonid community studied here is thus not very specific as it is composed of species that can be found in the area in phytal and other hard bottom communities of the upper infralittoral. Noteworthy is the fact that in our study we have quantitative data not only for adults but also for juveniles which made up about 10% of the total amount of pycnogonid specimens. This does not seem to be exceptionally high, since Esquete *et al.* (2013) found a much higher proportion of juveniles that varied seasonally in seagrass beds.

In our view, an interesting aspect of this study is that the rug-like habitus and belt-like distribution of *Corallina officinalis* allow quite easily for quantitative analysis of pycnogonids, and also other *Corallina*-inhabiting organisms (Buršić *et al.*, 2019), from samples of standardized size. In most other Mediterranean pycnogonid habitats, this is more difficult due to the growth type and distribution of the habitat-forming organisms since they extend way more into the water column, are often composed of multiple long branches, and thus have a strongly varying surface where pycnogonids may settle. Krapp-Schickel & Krapp (1975) have therefore recorded precisely whether whole macroalgae or parts (terminal branches, basal portions) were harvested in their analysis of phytal communities at the Banjole island, ca 30 km north of our sample localities. Esquete *et al.* (2013) have used Van Veen grabs to study pycnogonid communities of seagrass meadows in the east Atlantic. This method makes sure that sampling bias is minimized in a similar way as has been done in the present study by complete removal of a standardized sample area. In recent zoogeographic analyses, the habitat ties of the pycnogonid species have not been considered (Chimenz Gusso & Lattanzi, 2003; Soler Membrives & Munilla, 2015), since most data about Mediterranean pycnogonids is based on the usual hand sampling methods or dredging.

Conversely, quantifiable samples, allow one to perform statistical analyses of the distribution of the pycnogonid species, as we did here for exposed low impact and sheltered high impact *Corallina* communities showing that pycnogonids in general, and also most of the species, are more common at the exposed low impact sites than at the other sites. Only *Tanystylum conirostre* proved to be

a species ubiquitous in both *Corallina* habitat subtypes.

Hence, the combination of an exposed site with low human impact results in a higher pycnogonid abundance than the other sites studied. It is not possible, however, to decide whether exposition or human impact are the main factors for the pycnogonid abundance since all exposed sites are also low impact sites and all sheltered sites except one are high impact sites. The two factors are thus not separable. In fact, the species composition is nearly the same: the one on “high impact and sheltered” is a subset of “low impact and exposed”. However, it’s interesting to note that these differences occur even though the pycnogonid communities in *Corallina* belts are composed of species of low rather than a high degree of habitat association.

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## Supplementary data

The following supplementary information is available online for the article:

**Table S1:** Sample localities and pycnogonid species identified from the samples (5 cm<sup>2</sup> of *Corallina* each). Samples in which no pycnogonids were found are not shown.