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## Pycnogonids (Arthropoda: Pycnogonida) of Portofino, Ligurian Sea (North-Western Mediterranean Sea)

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#### Abstract

Pycnogonida collected monthly from September 2017 to August 2018 in the Portofino Marine Protected Area at 0-5 m depth were studied. A total of 499 specimens were collected, 457 of which were identified to species level. These were classified as belonging to 10 species: Achelia echinata\*, Ascorhynchus castelli, Neotrygaeus communis\*, Tanystylum conirostre\*, Anoplodactylus angulatus, A. petiolatus, A. pygmaeus\*, A. virescens, Callipallene phantoma and C. tiberi\*. For five dominant species (those marked with an asterisk) the annual phenology was outlined. Four hundred and seventeen additional specimens, collected from the same area and depth range mainly during the 1970s and 1980s were identified to species level for completeness of information, leading to the addition of Pycnogonum pusillum and Endeis spinosa.

**Keywords:** Pantopoda, Mediterranean, annual cycle

#### Introduction

The Pycnogonida is a poorly known class of marine arthropods represented by 1350 species described (Bamber et al. 2019). Lehmann et al. (2014) report that 56 species are known for the Mediterranean Sea, consisting mostly of littoral species, while deep-sea ones are an exception. This is assumed to be due to geological and temperature barriers that isolate Mediterranean deep water and make its fauna relatively species-poor Lehmann et al. 2014). The Western Mediterranean fauna is better known: the Iberian and the Balearic waters host 36 species (Soler-Membrives & Munilla 2015), 37 species are present in the French part (Arnaud 1987) and 45 are those recorded in the Italian seas (Bartolino & Chimenz 2010). For the Eastern Mediterranean, there are less studies published and the number of species known is lower: for example, in the Turkish waters, only 11 species are recorded (Koçak 2013). Few cases of in-depth research on the Pantopoda of a specific area are known in the Italian literature and they are all focused on South-Central and Southern Italy (Krapp 1973; Chimenz Gusso et al.

1978; Chimenz et al. 1979, 1993; Chimenz & Cottarelli 1986; Piscitelli & Barone 2000). In the Ligurian Sea, mainly based on sporadic collections (Faraggiana 1940; Krapp 1975; Arnaud 1987; Chimenz Gusso 2000), 17 species have been recorded (Bartolino & Chimenz 2010).

This paper is based on data gathered in the Portofino Marine Protected Area, Ligurian Sea, during a recent continuing benthic biodiversity monitoring project. Data based on the identification of specimens from unstudied collections made in the same area during the 1970s and 1980s are incorporated in this report.

#### Materials and methods

Study area

The Portofino Marine Protected Area (MPA) covers a total area of 346 ha in the eastern Ligurian Sea (northern Mediterranean Sea), and is mainly characterized by conglomerate vertical underwater faces up to 60 m deep (Tortonese 1958, 1961). In recent

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years, some benthic taxa collected from these faces have been studied, i.e. macroalgae (Mangialajo et al. 2004), sponges (Pansini & Pronzato 1990; Pansini & Musso 1991; Pronzato et al. 1998) and cnidarians (Boero et al. 1986; Puce et al. 2009; Bavestrello et al. 2015). Some studies, driven by mass mortality following water warming events (e.g. Cerrano et al. 2000; Schiaparelli et al. 2007; Cerrano & Bavestrello 2008), focused on the long-term changes in structure and composition of the benthic communities (Bertolino et al. 2016; Betti et al. 2017; Longobardi et al. 2017).

#### Materials and methods

The sampling area extended for about 300 m along the vertical eastern side of Punta del Faro, the southeastern cape of the Portofino Promontory (WGS 84 coordinates of the sampling site: 44°17′55.70"N, 9° 13'7.15"E), at depths between the sea surface and 5 m. Sampling activities, conducted monthly between September 2017 and August 2018, were undertaken using SCUBA. Main macroalgae (Ellisolandia elongata, Flabellia petiolata, Dyctiota dichotoma) and hydroids (extremely variable along the year, but mainly Eudendrium spp., Aglaophenia spp., Halecium spp., Sertularella ellisii) were randomly collected on the vertical rocky cliff by visually oriented sampling. In total, around 113 g (standard error: 10.97) of organisms, covering a standard surface of about 900 cm<sup>2</sup>, were collected every month.

Samples were fixed in formaline 4% and carefully sorted using a stereomicroscope. The pycnogonids were transferred to ethanol 70% for further analysis.

Additional still unstudied material collected from the same area mainly during the 1970s and 1980s was identified to species level for completeness of information. In particular, we limited our analysis to samples collected at a maximum depth of 15 m compatible with the bathymetric range of the species more linked to surface waters among those identified in the recent collections (0–13 m in *Tanystylum conirostre* – see Munilla & Soler-Membrives 2014).

Identification of specimens was mainly based on descriptions of the Iberian fauna reported in Munilla and Soler-Membrives (2014). For some details (e.g. the strigils spine formula) and diagnostic measurements an interference contrast microscope (Leica DM LB2), a Leica DFC 295 camera and Leica Application Suite (Vers. 3.8) was used.

All the studied material is conserved in the collection of the authors at the Department of Earth, Environment and Life Sciences (DISTAV) of Genoa University.

For the five dominant species collected between September 2017 and August 2018 and in the additional material, the annual phenology was outlined, based on the monthly presence of males carrying egg clusters, adults of both sexes and juveniles or subadults. Using these and additional data based on previous collections, the sex ratio (number of males/number of females) was determined. The statistical significance of the differences from the expected value (1) of the species sex ratio was assessed via the Chi-square test, using PAST software (Hammer et al. 2001; Vers. 3.22, last accessed December 2018).

#### Results

A total of 499 pycnogonids were collected, with a period of near constant abundances from summer to mid-winter and then an increase leading up to a peak abundance during spring (Table I). Just 42 juveniles specimens were unidentified (or identified to genus level); the other 457 were classified to species level and assigned to 10 species: Achelia echinata Hodge, 1864, Ascorhynchus castelli (Dohrn, 1881), Neotrygaeus communis (Munilla & Alonso-Zarazago, 2014), Tanystylum conirostre (Dohrn, 1881), Anoplodactylus angulatus (Dohrn, 1881), A. petiolatus (Kröyer, 1884), A. pygmaeus (Hodge, 1864), A. virescens Hodge, 1864, Callipallene phantoma (Dohrn, 1881) and C. tiberi (Dohrn, 1881) (Table I; Figure 1).

These species are known to be generally observed on algae, sponges, bryozoans and hydrozoans (see Munilla & Soler-Membrives 2014). The bathymetric range is narrow in *N. communis* (0–24 m), *T. conirostre* (0–13 m), *A. angulatus* (0–35 m), *A. virescens* (0–40 m), and wide in the other six species (0–537 m in *A. echinata*, 0–130 m in *A. castelli*, 0–1500 m in *A. petiolatus*, 0–587 m in *A. pygmaeus*, 0–850 m in *C. phantoma* and 0–523 m in *C. tiberi*). Moreover, *A. angulatus*, *A. pygmaeus* and *A. virescens* larvae are parasites of hydroids, and those of *A. petiolatus* can also be found on the manubrium or the bell of some jellyfish (see Chimenz Gusso 2000; Munilla & Soler-Membrives 2014).

For the five dominant species, the annual phenology is outlined (Figure 2). Males of A. echinata, T. conirostre and A. pygmaeus with egg clusters were recorded twice during the year; they appeared three times in C. tiberi and just once in N. communis. These observations suggest that these species are bivoltine, trivoltine and univoltine, respectively.

One female of *N. communis* collected in March 2018 was abnormal with the two central

	2017				2018							
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Achelia echinata	2	1	2	6	4		4	5	17	12		3
Ascorhynchus castelli			1	5	1	6	9	16	1	3		
Neotrygaeus communis	3	1	1		9	4	24	16	7	2	1	2
Tanystylum conirostre	1	3	2	1		20	23	23	10	9	2	2
Anoplodactylus angulatus				1								
Anoplodactylus petiolatus				1								
Anoplodactylus pygmaeus			1	1	1	4	26	23	27	6	9	
Anoplodactylus virescens							11					
Callipallene phantoma						1	2					
Callipallene tiberi	10	5	14	9	2	5	2	9	16	5	1	1
Total	16	10	21	24	17	40	101	92	78	37	13	8

Table I. Monthly abundances of Pycnogonida species in Portofino (0-5 m depth) between September 2017 and August 2018.

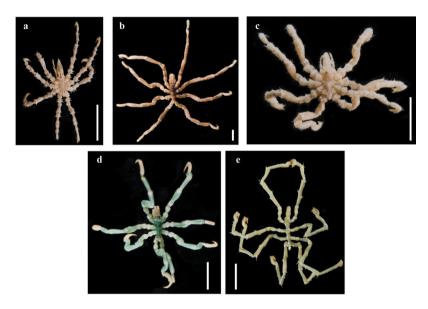


Figure 1. a, Achelia echinata; b, Neotrygaeus communis; c, Tanystylum conirostre; d, Anoplodactylus virescens; e, Endeis spinosa. Scale bars: 2 mm.

segments of the trunk modified and mutually arranged in an atypical manner and with three pairs of legs (Figure 3).

The inclusion of an additional 417 specimens confirmed the presence in the study area of nine of the species mentioned above: only *A. virescens* is absent in such material. However, among these pycnogonids we identified two species which we could add to the current list (Table II): *Pycnogonum pusilum*, a species abundant amongst algae from 0.5 to 35 m, and *Endeis spinosa* (Figure 1e), a species found on *Posidonia oceanica* and algae at depths from 0 to 100 m (see Munilla & Soler-Membrives 2014). Larvae of this species are parasites of polyps of hydroids and jellyfish (see Chimenz Gusso 2000). In Table III the phenology of the species based on the additional material is shown.

Sex ratio determined for the five dominant species is shown in Table IV: in three of them (*T. conirostre*, *A. pygmaeus* and *C. tiberi*) a statistically significant predominance of females was recorded.

#### Discussion

The upper infralittoral zone of Portofino hosts a rather rich benthic fauna (e.g. Bertolino et al. 2016; Betti et al. 2017; Longobardi et al. 2017) and this is supported by our study of the Pycnogonida. The number of species identified in our study is close to that recorded by Chimenz et al. (1979) in the Civitavecchia harbor in the Tyrrhenian Sea.

Two papers report on the collection of pycnogonids from Portofino. Faraggiana (1940) recorded four species from very shallow waters: *Callipallene* 

Ac.ec.	Ad	Meggs	Juv	Ne.co.	Ad	Meggs	Juv	Ta.co.	Ad	Meggs
Jan				Jan				Jan		
Feb				Feb				Feb		
Mar				Mar				Mar		
Apr				Apr				Apr		
May				May				May		
Jun				Jun				Jun		
Jul				Jul				Jul		
Aug				Aug				Aug		
Sep				Sep				Sep		
Oct				Oct				Oct		
Nov				Nov				Nov		
Dec				Dec				Dec		
		An.py.	Ad	Meggs J	uv	Ca.ti.	Ad	Meggs .	luv	
		Jan				Jan				
		Feb				Feb				
		Mar				Mar				
		Apr				Apr				
		May				May				
		Jun				Jun				
		Jul				Jul				
		Aug				Aug				
		Sep				Sep				
		Oct				Oct				
		Nov				Nov				
		Dec				Dec				

Figure 2. Phenology of five dominant species of sea spiders in Portofino (September 2017–August 2018): Ac.ec. = Achelia echinata; Ne.co. = Neotrygaeus communis; Ta.co. = Tanystylum conirostre; An.py. = Anoplodactylus pygmaeus; Ca.ti. = Callipallene tiberi. Ad = adults; Meggs = males with eggs clusters; Juv = Juveniles.

brevirostris (probably C. spectrum, based on the taxonomical remarks of Chimenz Gusso 2000), Ammothea longipes, Α. uniunguiculata A. echinata. Arnaud (1987) reported on three species: T. conirostre, N. communis, A. pygmaeus. Comparing our data to the list of species of the Ligurian Sea (Area 1) in Bartolino and Chimenz (2010), eight species are missing in our collections: Ammothellaappendiculata (Dohrn, A. longioculata (Faraggiana, 1940), A. longipes (Hodge, 1864), Ascorhynchus arenicola (Dohrn, 1881), Paranymphon spinosus Caullery, 1896, Pycnogonum plumipes Stock, 1968, Endeis charybdaea (Dohrn, 1881), Nymphon gracile Leach, 1814. Pycnogonum plumipes is a sciaphilous species common on coralline bottoms and present also in submarine caves and was found by Arnaud (1987) near Savona (Western Liguria). All the others are species normally found in *Posidonia* prairies and/or on algae on sandy bottoms (see Chimenz Gusso 2000; Munilla & Soler-Membrives 2014). Two other species known from the Portofino area, Callipallene spectrum and A. uniunguiculata (Faraggiana 1940), were no longer detected. These species are not listed by Bartolino and Chimenz (2010) amongst Ligurian





Figure 3. Abnormal *Neotrygaeus communis* female specimen: dorsal (left) and ventral (right) view. Scale bar: 0.5 mm.

Sea Pycnogonida, probably due to an oversight. The former is collected on algae between 0 and 160 m depth, while the latter is associated to *Posidonia* prairies and algae from 2.9 to 16 m (Chimenz Gusso 2000; Munilla & Soler-Membrives 2014). On the other hand, on the vertical rocky cliff of Portofino shallow waters, we found *C. phantoma*, *C. tiberi* and *P. pusillum*, which are new records for the Ligurian Sea.

Table II. Additional material identified to species level.

Locality	Collection date	Depth (m)	Habitat	Collector	Species	Nr of specimens
Portofino "transept Aurora"	10.IV.1979	10–15		Bianchi &	Achelia echinata	1
•				Morri	Neotrygaeus communis	2
					Tanystylum conirostre	9
					Endeis spinosa	3
Portofino "transept Aurora"	11.V.1979	0		Bianchi &	Neotrygaeus communis	1
				Morri	Tanystylum conirostre	15
Portofino "transept Aurora"	11.V.1979	5–10		Bianchi & Morri	Tanystylum conirostre	2
Portofino "transept Aurora"	11.V.1979	10-15		Bianchi &	Achelia echinata	5
				Morri	Neotrygaeus communis	6
					Tanystylum conirostre	1
					Ascorhynchus castelli	2
Portofino Punta Chiappa	23.VI.1979	10–15		M. Mori	Achelia echinata	1
Portofino "transept Aurora"	26.VI.1979	1–5		Bianchi &	Neotrygaeus communis	3
ortonno transept Aurora	20. 11.1979	1-5		Morri	Tanystylum conirostre	3
Portofino Punta del Faro	10 IV 1070	0-15		M. Mori	Achelia echinata	4
ronolino runta del raro	18.IX.1979	0-15		IVI. IVIOII		
					Neotrygaeus communis	1
					Tanystylum conirostre	8
D 6 "				D: 1:0	Callipallene phantoma	1
Portofino "transept Aurora"	8.II.1980	0–5		Bianchi &	Neotrygaeus communis	3
				Morri	Tanystylum conirostre	19
					Callipallene tiberi	1
Portofino "transept Aurora"	8.II.1980	5–10		Bianchi &	Neotrygaeus communis	2
				Morri	Tanystylum conirostre	8
					Anoplodactylus petiolatus	1
Portofino "transept Aurora"	8.II.1980	10–15		Bianchi &	Achelia echinata	6
				Morri	Neotrygaeus communis	1
					Tanystylum conirostre	1
					Anoplodactylus angulatus	2
Portofino "transept Aurora"	18.III.1980	0		Bianchi & Morri	Tanystylum conirostre	35
Portofino "transept Aurora"	18.III.1980	0-5		Bianchi &	Achelia echinata	7
				Morri	Neotrygaeus communis	10
					Tanystylum conirostre	81
					Anoplodactylus petiolatus	1
					Callipallene tiberi	2
					Pycnogonum pusillum	1
					Endeis spinosa	3
Portofino "transept Aurora"	18.III.1980	5-10		Bianchi &	Achelia echinata	2
				Morri	Neotrygaeus communis	3
				1120111	Tanystylum conirostre	24
					Ascorhynchus castelli	1
Portofino "transept Aurora"	18.III.1980	10–15		Bianchi &	Achelia echinata	4
rortonno transcpt riarora	10.111.1700	10 15		Morri	Neotrygaeus communis	15
				WIOIII	Tanystylum conirostre	3
					Anoplodactylus pygmaeus	1
Portofino "transept Aurora"	VI.1980	5		M. Mori	Achelia echinata	1
rortoillo transept Aurora	V1.1960	,		IVI. IVIOII	Neotrygaeus communis	1
					20	5
					Tanystylum conirostre	
Domtofino Domos-:	7 VI 1000	7		Diam s1-: 0-	Ascorhynchus castelli	1
Portofino Paraggi	7.VI.1980	7		Bianchi &	Achelia echinata	1
				Morri	Ascorhynchus castelli	1
D . C	10 137 1000	2	0 . 1 . 1		Endeis spinosa	1
Portofino "transept Aurora"	13.IX.1980	3	On tethered	M. Mori	Achelia echinata	2
			panel		Neotrygaeus communis	2
					Tanystylum conirostre	2
Portofino "transept Aurora"	17.XI.1980	10–15		F. Boero	Endeis spinosa	1
Portofino	Summer 1980	3		Bianchi & Morri	Tanystylum conirostre	3

(Continued)

Table II. (Continued).

Locality	Collection date	Depth (m)	Habitat	Collector	Species	Nr of specimens
Portofino "transept Aurora"	12.VII.1983	3–15	On algae	M. Mori	Achelia echinata	6
					Neotrygaeus communis	2
					Tanystylum conirostre	3
					Callipallene phantoma	1
					Callipallene tiberi	1
					Endeis spinosa	3
Portofino "transept Aurora"	12.IX.1983	3	On tethered	M. Mori	Achelia echinata	1
			panel		Neotrygaeus communis	1
					Tanystylum conirostre	3
Portofino "transept Aurora"	IX.1983	10	On tethered panel	Bianchi & Morri	Achelia echinata	1
Portofino "transept Aurora"	X.1984	5	On tethered	Bianchi &	Achelia echinata	1
			panel	Morri	Ascorhynchus castelli	1
					Endeis spinosa	2
Portofino Paraggi	3.III.1988	4	On algae	Bianchi &	Achelia echinata	2
55			Ü	Morri	Tanystylum conirostre	1
Portofino Paraggi	9.III.1988	7		Bianchi &	Achelia echinata	3
				Morri	Tanystylum conirostre	1
Portofino Paraggi	7.VI.1988	4–7	Rocky seabed	Bianchi &	Achelia echinata	3
			-	Morri	Neotrygaeus communis	3
					Tanystylum conirostre	1
					Callipallene tiberi	4
					Endeis spinosa	1
Portofino Paraggi	7.IX.1988	4	Rocky seabed	Bianchi &	Achelia echinata	5
			•	Morri	Tanystylum conirostre	2
					Anoplodactylus angulatus	1
Portofino Paraggi	21.XII.1988	4–7	Rocky seabed	Bianchi &	Achelia echinata	5
			•	Morri	Anoplodactylus angulatus	2
					Callipallene tiberi	2
Portofino S. Giorgio–Punta del	20.V.2016	0-5	On Hydrozoa	F. Betti	Achelia echinata	2
Faro			· ·		Neotrygaeus communis	2
					Tanystylum conirostre	1
					Anoplodactylus petiolatus	2
					Callipallene phantoma	1
					Callipallene tiberi	2
Portofino S. Giorgio–Punta del Faro	13.IX.2016	0–5	On Hydrozoa	F. Betti	Callipallene phantoma	3
Portofino	20.VII.2018	10	On buoy	F. Betti	Achelia echinata	2
			j	–	Ascorhynchus castelli	2
Portofino S. Giorgio–Punta del	IX.2018	0–5		F. Betti	Achelia echinata	2
Faro		0 3			Tanystylum conirostre	4
					Anoplodactylus pygmaeus	1
					Callipallene tiberi	5

The phenologies recorded for five dominant species differ at least partially from those known from literature for the same species (Munilla Leôn 1980; Chimenz Gusso 2000), possibly indicating an influence of local and annual climatic conditions on their reproductive activity. This hypothesis seems to be confirmed also by the difference between the phenologies shown in Figure 1 and data about the same species in Portofino based on additional material (Table III).

The sex ratio in favor of females evidenced in some species may be due to the particular parental care system of pycnogonids, where males carry multiple groups of eggs gathered from multiple females simultaneously (Arnaud & Bamber 1987). However, *Achelia echinata* males were slightly more abundant suggesting other influences.

Anoplodactylus angulatus postlarva is known to be a parasite of hydroids belonging to genus *Tubularia* (King 1973). No species of the genus *Tubularia* were found in the area during the sampling period, but they are known to be present both deeper on the same cliff face (Puce et al. 2009) and in the

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Achelia echinata		A*J	A*J	A	AJ	AJ	A		A*J	A*		AJ
Ascorhynchus castelli		3	A		Å	Å	A*		,			3
Neotrygaeus communis		AJ	AJ	AJ	AJ	Α			Α			
Tanystylum conirostre		A*J	A*J	AJ	AJ	Α	Α		A*J			
Anoplodactylus angulatus		A							J			A
Anoplodactylus petiolatus			Α		A				-			
Anoplodactylus pygmaeus			Α						A			
Callipallene phantoma					AJ		A*J		A			
Callipallene tiberi		A	Α		J	A*	AJ		A			A
Pycnogonum pusillum			Α		-		-					
Endeis spinosa			J	A		A	AJ			A	Α	

Table III. Monthly phenology of Pycnogonida species in Portofino based on additional data (specimens collected mainly during the 1970s and 1980s; 0-15 m depth). A = Adults,  $A^*$  = males with eggs, J = Juveniles.

Table IV. Sex ratio of five dominant species collected in Portofino. M = number of males, F = number of females, Sex ratio = M/F,  $\chi^2$  1df, P(same) and Monte Carlo P(same) = chi-square test value to assess the statistical significance of the differences from 1 of the sex ratio and relative probability of identity (also corrected based on the Monte Carlo method).

	M	F	Sex ratio	$\chi^2$ 1df	P(same)	Monte Carlo P(same)
Achelia echinata	48	34	1.41	2.39	0.12	0.15
Neotrygaeus communis	23	36	0.560976	2.86	0.09	0.119
Tanystylum conirostre	37	105	0.367925	32.563	<<0.01	0.0001
Anoplodactylus pygmaeus	28	46	0.61	4.38	0.04	0.048
Callipallene tiberi	27	53	0.51	8.45	0.004	0.006

surrounding waters: for example, along a buoy chain less than 50 m distant from the survey area.

We recorded a malformation in an *N. communis* specimen with three segments and as many pairs of legs. Such abnormalities are not unknown in pycnogonid development and they are mainly attributable to errors in the regeneration of appendages and other body parts after being injured (except for some cases of gynandromorphism - Scholtz & Brenneis 2016).

#### Conclusions

Pycnogonids are poorly known arthropods. The preliminary results of our study of the upper infralittoral zone fauna at Portofino add to the knowledge of these animals in Italy, both as regards the geographical distribution of species identified and their phenology. It was possible to identify 12 species, three of which not previously known in the Ligurian Sea (C. phantoma, C. tiberi and P. pusillum), and for five of them (A. echinata, N. communis, T. conirostre, A. pygmaeus and C. tiberi) the annual cycle was outlined.

The continuation of this research will hopefully provide further knowledge to the species richness of the Portofino area and, thanks to future collections, could also allow us to extrapolate more detailed information on the life cycles of a larger number of species. Moreover, our purpose is to collect material also in the close *Posidonia oceanica* prairie to obtain data on the species related to this habitat.

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#### Disclosure statement

No potential conflict of interest was reported by the authors.

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