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Research Article

Characterization of the first invasive population of sea spiders (Pycnogonida)

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Abstract

Pycnogonids, or sea spiders, are ubiquitous arthropods present in most marine ecosystems. However, because of their usually low population densities, they remain understudied by zoologists despite presenting numerous unique biological features. The species *Ammothea hilgendorfi* (Böhm, 1879), native to the Northern Pacific Ocean, was introduced in Europe in the late 1970s and its presence has now been reported in several seas. Recordings of the species in Europe are sporadic and mostly do not exceed a handful of specimens. Hence, the populations have only been considered as introduced in the invasion process. The goal of this study is to investigate the first population of *A. hilgendorfi* at the Belgian coast (North Sea) during a 24-month monitoring through different proxies (phenology, biometry, developmental cycle). For the first time, our results demonstrate that this population of pycnogonids can be considered as established as it is able to complete its entire life cycle, including all larval instars, which are ectoparasites of hydroids. Moreover, it has developed in very high densities of individuals and has outnumbered by far the native sea spiders, which have almost disappeared, so it can also be considered as invasive *sensu stricto*. Their average density increased from the first year to the second, which indicates that the invasion has likely not reached its capacity yet. Since research on sea spiders has been very limited, this work emphasizes the importance of studying these animals, especially when the lack of knowledge undermines the assessment of their impact and their invasiveness potential.

Key words: larva, monitoring, alien, life cycle, Ammotheidae, Pantopoda, phenology

Introduction

Pycnogonids, also called sea spiders or pantopods, are marine arthropods distributed globally, represented by around 1400 extant described species (Bamber et al. 2024). They remain poorly investigated by zoologists, notably due to their usually low population densities (Sabroux et al. 2019). Most samplings occur alongside other organisms in large-scale expeditions or are part of wider fauna inventories (e.g. Blankley and Grindley 1985; Perez-Ruzafa and Munilla 1992; Cano-Sánchez and López-González 2018; Sabroux et al. 2019; Fornshell 2023). Sea spiders are seldom the main object of sampling or dedicated studies, especially at a population scale (a few examples are Wilhelm et al. (1997) and Lovely (2005)). Nonetheless, they remain unique model organisms to study, partially because of their morphology: a trunk reduced to the point that their reproductive and digestive tracts extend into the legs (Arnaud and Bamber 1988), which can be autotomized and grow back via successive molts (Fornshell 2019; Flandroit et al. 2024); a pair of specialized ventral limbs, the ovigerous legs, used by males to carry the eggs (i.e. exclusive paternal care) (Bain and Govedich 2004); and the tubercle, a periscope-like structure mainly carrying peculiar sensory organs (Lehmann et al. 2018). Their life cycle is also singular: the eggs hatch as protonymphon larvae, which can be regarded as the "future adult head" at the end of which the trunk segments appear in successive molts (Brenneis et al. 2017). In many species, the larvae are parasitic of organisms such as sessile cnidarians (Brenneis et al. 2017). The position of this group in the arthropod evolutionary tree is also hazy (Arango and Wheeler 2007; Giribet and Edgecombe 2019), but the current consensus considers sea spiders as basal chelicerates, as the sister group to euchelicerates (which includes arachnids and xiphosurans) (Arango and Wheeler 2007; Giribet and Edgecombe 2019).

Ammothea hilgendorfi (Böhm, 1879) (Ammotheidae), called "zebra zeespin" (zebra sea spider) in Dutch or シマウミグモ ("shima umigumo"; striped sea spider) in Japanese is quite a common species recognizable by its atrophied and rounded chelifores bearing a spine at the distal end, and well distinct lateral processes on the trunk (Bamber 2010; Faasse 2013). The first three trunk segments show a posterior ridge dorsally (Faasse 2013). It also displays a unique color pattern: a yellowish body spotted with reddish-brown, and a brownish "V" on the proboscis (Le Roux et al. 2020). While it was historically collected on littorals off the North Pacific Ocean (e.g. Sagami Bay, Kanagawa, Japan (Utinomi 1951) but see Miyazaki (2022) for a complete list of records; California, USA (Bain 1991; Barreto and Avise 2008)), it can now be encountered in Europe. Its introduction in the Mediterranean Sea was attributed to biofouling on ship hulls arriving from East Asia via the Suez Canal (Krapp and Sconfietti 1983). It is also thought to have traveled to the UK through maritime commercial shipping routes (Bamber 2012; Faasse 2020). Table 1 summarizes every preceding significant recording of *A. hilgendorfi* in Europe.

Following its introduction in Europe, *A. hilgendorfi* has been referred to by various denominations such as "adventitious" or "immigrant" (respectively, Krapp and Sconfietti 1983; Bamber 2012). This lack of consensus and homogeneity in the terminology is common to many other cases of biological invasions and a fair number of other terms are used to characterize alien species. Several authors such as Colautti and MacIsaac (2004) and Blackburn et al. (2011) hence proposed a unified terminology to characterize bioinvasions – here considered as the main references to intepret this study. They described the process of biological invasion as a series of steps and filters that a species must successively overcome to go from native to invasive. First, it must

Table 1. Recordings of the European populations of *Ammothea hilgendorfi* previously described in the literature. Sampling data refer to the quantitative information mentioned in the corresponding references.

Year	Location	Country	Sampling data	Reference
1978	Southampton Water, Hampshire	England	2 juveniles	Bamber 1985
1979–1981	Venice Lagoon	Italy	6 females and 5 juveniles over 5 collection periods	Krapp and Sconfietti 1983
1987	Southampton Water	England	1 adult	Bamber 1988
1991	Venice Lagoon	Italy	4 females and 1 male	Mizzan 2018
2001–2006	Poole Harbor, Dorset	England	“Large numbers”	Bamber 2012
2009	Poole Bay, Dorset	England	NA	Bamber 2012
2010	West Solent	England	NA	Bamber 2012
2010	Essex	England	20 specimens	Bamber 2012
2010	Mersin Bay	Turkey	1 female and 1 juvenile	Ragkousis et al. 2020
2010–2011	Southampton Water, Hampshire	England	“Numerous records”	Bamber 2012
2013	Zierikzee	Netherlands	17 specimens	Faasse 2013
2016–2017	Venice Lagoon	Italy	24 specimens (including juv. and ovigerous males) over 8 stations and 4 collection periods	Mizzan 2018
2019	Etel	France	70 specimens (including juv. and ovigerous males)	Le Roux et al. 2020
2020–2021	Rance Maritime	France	Several specimens, punctual densities up to 15/dm²	Le Roux et al. 2022
2021	Golf of Morbihan	France	19 specimens over two collection periods	Le Roux et al. 2022
2022	Saint-Malo	France	One specimen	Le Roux et al. 2022
2022	Pénestin	France	NA	Le Roux et al. 2022

access and survive a pathway to be transported to a new area (i.e. introduced). Second, the introduced population must survive and complete its life cycle (i.e. established). Third, the population must become locally dominant and/or spread out of its introduction area (i.e. invasive). Other terms such as “alien”, “non-native” or “non-indigenous” are more general and cover all the stages described here above.

Ammothea hilgendorfi is an introduced species in Europe and is categorized as “alien” (i.e. introduced) in the European Alien Species Information Network (European Commission 2024). Indeed, despite the presence of eggs and juveniles in some studies and suspicions of establishment (e.g. Krapp and Sconfietti 1983; Bamber 1985; Mizzan 2018; Le Roux et al. 2020), no larvae nor any life cycle completion were reported yet, therefore evidence to consider *A. hilgendorfi* as established, let alone invasive, is lacking. Four other alien sea spider species were reported in Europe and neighboring countries but not with the same extent as *A. hilgendorfi* whatsoever (European Commission 2024): *Achelia sawayai* s.l. (Marcus, 1940) recorded in Italy and Malta in 2016 (Ulman et al. 2017); *Anoplodactylus californicus* (Hall, 1912) recorded in Egypt in 1936 (Helfer 1936); *Anoplodactylus digitatus* (Böhm, 1879) recorded in Israel in 1951 (Stock 1958); and *Pigrogromitus timsanus* (Calman, 1927) recorded in Israel in 1960 (Lipkin and Safriel 1971). Other records of non-native pycnogonids globally include *Endeis spinosa* (Montagu, 1808) and *Pycnogonum cessaci* (Bouvier, 1911), found off the coast of Argentina, with an uncertain origin (i.e. cryptogenic species) (Schwindt et al. 2020); and *Anoplodactylus monotrema* (Stock, 1979), reported as alien in the Galápagos (Carlton et al. 2019). Among these species, none has gone beyond the status of introduced, as recordings are scarce and do not surpass a handful of specimens.

Within its native range, studies focused on *A. hilgendorfi* are scarce. Among the few ones can be mentioned Barreto and Avise (2008), who described the species to be polygynandrous, meaning that both males and females mate several times with different partners in a single reproduction period. Observations were made of associations of *A. hilgendorfi* with holothurians by Ohshima (1927) and sea stars by Nakamura and Fujita (2004), who also described the juvenile development. The life cycle begins with protonymphon larvae hatching on the male ovigers and quickly leaving their progenitor (Bain 1991) to parasitize hydroids and develop into free-living juveniles (Russel and Hedgpeth 1990). Interestingly, the larval development of *A. hilgendorfi* was described as endoparasite, despite the first instar lacking the typical endoparasitic features (Brenneis et al. 2017). It was the first and only ammotheid identified as an endoparasite and the only pycnogonid confirmed to show that specific postembryonic pathway.

The Belgian pycnogonid fauna was inventoried on the wave breakers in Knokke (West Flanders, Belgium) in the second half of the past century (Daro 1969, 1970). Three sea spider species were reported: *Pycnogonum litorale* (Strøm, 1762), *Nymphon brevirostre* (Hodge, 1863) and *Phoxichilidium femoratum* (Rathke, 1799). Since its introduction in Europe, records of *A. hilgendorfi* in Belgium were very limited. The Vlaams Instituut voor de Zee (VLIZ) Alien Species Consortium reported two specimens found in 2013 at 31 meters deep, 30 kilometers offshore (Faasse 2020; Flandroit and Faasse 2024). The World Register of Introduced Marine Species (WRiMS) reported *A. hilgendorfi* in 2017 (Costello et al. 2024). According to the Global Biodiversity Information Facility (GBIF), three specimens were found, at the earliest on 17 April 2022 (GBIF Secretariat 2023). Since then, a few observations have been reported in citizen science databases such as Observation.org or iNaturalist.

The first aim of the present study is to investigate the first population of *A. hilgendorfi* at the Belgian coast (North Sea) during a 24-month monitoring using a robust scientific strategy by measuring different proxies, such as their density and infestation rate on oysters, their abundance relative to other sea spiders species, the ratio of adult males, females, and juveniles, the presence of eggs on males, and the trunk length and width of the collected individuals. The second aim of this research is to demonstrate that this population is well established in Belgium by highlighting the presence of all the larval stages, then invasive by matching it with the dedicated criteria.

Materials and methods

Collection

From February 2022 to January 2024, monthly collections were carried out on rocky wave breakers in Knokke, West Flanders, Belgium (Figure 1A–D),

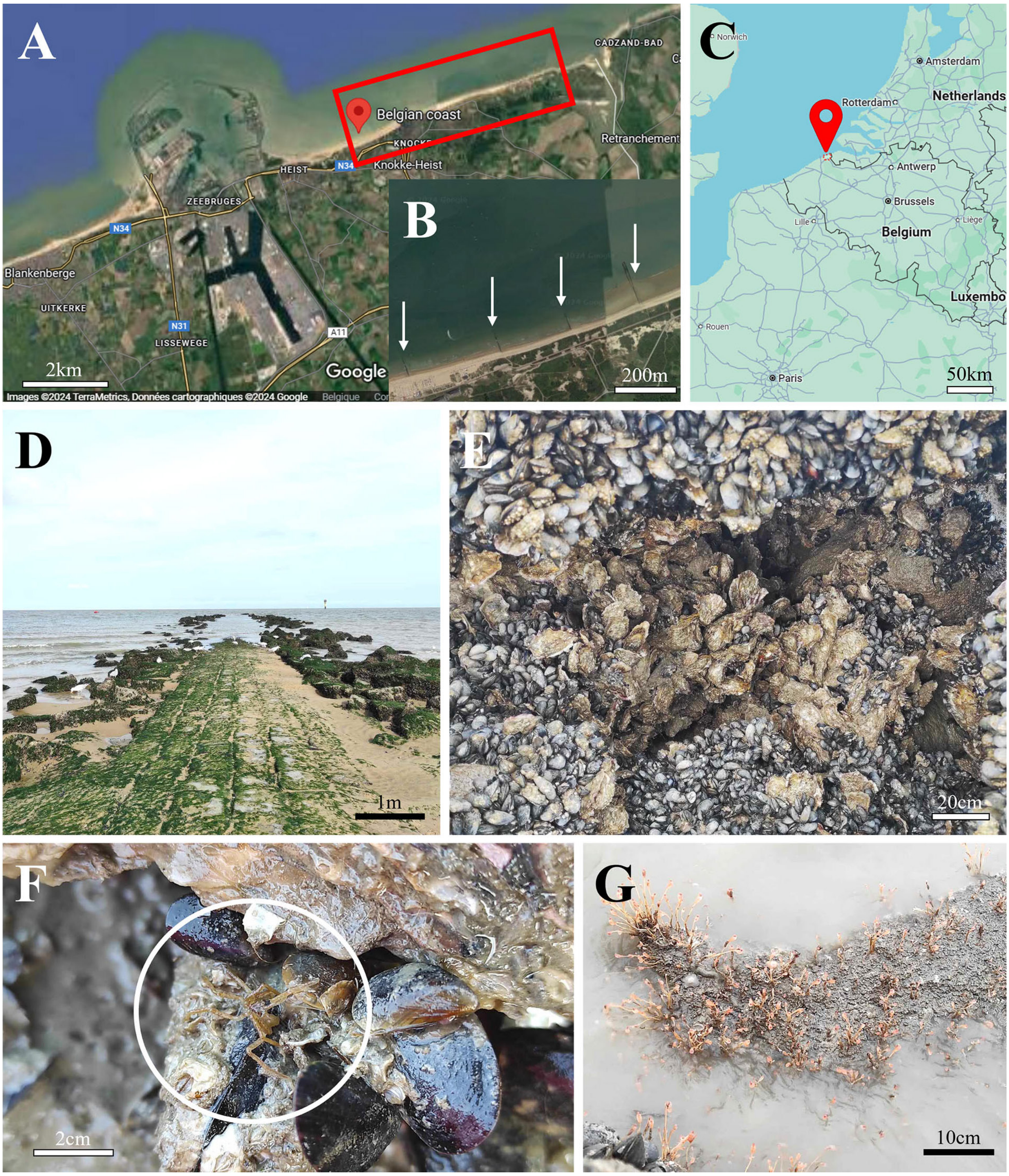

Figure 1. Sampling area. A: Satellite view of the sampling zone in Knokke-Heist (West Flanders, Belgium), inside the red rectangle, adapted from Google Maps (2024); B: Zoom on some of the wave breakers (white arrows) partially immerged on the image, adapted from Google Maps (2024); C: Map of the geographical position of the sampling area, Knokke-Heist (pointed in red), adapted from Google Maps (2025); D: One of the wave breakers at rising tide; E: Patches of oysters *Magallana gigas* surrounded by patches of mussels *Mytilus edulis*; F: Individual of *Ammothea hilgendorfi* (circled in white) walking on a *M. edulis*, itself attached to a *M. gigas*; G: rock covered with *Ectopleura larynx* hydroids (at the edge of a wave breaker). Photographs D–G ©Antoine Flandroit.

on mornings with high tide coefficients to maximize the time during which the rocks emerged (see Supplementary material Table S1: metadata of the collecting events including coordinates and tide ranges). These wave breakers, except for the most proximal part, are propitious for biofouling and are nowadays covered with reefs of the Japanese oysters *Magallana gigas*

(Thunberg, 1793) and the blue mussels *Mytilus edulis* (Linnaeus, 1758) (Figure 1E), which both shelter many species, including sea spiders. Preliminary collections revealed that *A. hilgendorfi* was mainly associated with the oyster heaps. Since it is cryptic and thereby hard to spot in the field, it was indirectly collected via *M. gigas*: a defined surface of oysters (see Table S1 for details) was randomly chosen on a random wave breaker, then sampled to fill a volume of 40 L, and brought back to the Biology of Marine Organisms and Biomimetics unit of the University of Mons (UMONS), Belgium. There, they were counted and meticulously investigated. Isolated oysters and clusters of merged oysters were both considered as "one oyster" because both were considered to represent a single ecological individual from the perspective of an epibiont such as a pycnogonid. All native pycnogonids hiding among the oyster samplings were counted, isolated, and identified to the species based on Hayward and Ryland (1995). The non-native *A. hilgendorfi* was identified based on the criteria in Bamber (2010) and Faasse (2013).

Population dynamics

Several parameters were surveyed. First, the relative abundance of each sea spider species was monitored. Based on the criteria in Nakamura and Fujita (2004), juveniles *A. hilgendorfi* were distinguished from adults; and among adults, males from females. The presence of ovigerous males (i.e. eggs on ovigerous legs) was also tracked. The density of *A. hilgendorfi* (ind/m^2) was obtained by dividing the number of specimens collected by the surface of the sampled area. The number of oysters was counted to assess the average number of *A. hilgendorfi* per oyster (i.e. infestation rate). For each month, binomial tests were processed to calculate the confidence interval (confidence level of 95%, or CI95; alpha = 5%) of several ratios. The sex ratio was obtained by dividing the total number of males by the total number of adults. Whenever the CI95 was strictly outside of 50% of males, the sex ratio was considered unbalanced. The juvenile ratio was obtained by dividing the number of juveniles by the total number of *A. hilgendorfi* specimens. Since adults remained abundant even when juveniles developed, it was arbitrarily considered as a juvenile peak whenever the CI95 included 50%. The ovigerous male ratio was obtained by dividing the number of egg-carrying males by the total number of males. It was considered as a reproduction peak whenever the CI95 was in majority above 50%, which is a less strict condition, because the smaller pool of organisms considered would obviously increase the size of the CI. All statistical analyses described herein above were performed using Rstudio 2023.12.1+402 (Rstudio Team 2020).

Biometric measurements

All *A. hilgendorfi* (except for 15 specimens in 2023) were photographed dorsally next to a reference scale using an Olympus TG-7 Tough camera.

Their lengths from the chelifore insertion to the 4th lateral process and widths between both 2nd lateral processes (i.e. same reference points as in Nakamura and Fujita (2004)) were measured using ImageJ (Schneider et al. 2012). To determine if the population biometry remained constant, a parametric t-test was performed to compare all lengths of the first 12 months (from now on referred to as "2022," despite including January 2023) with their counterparts of the last 12 months (i.e. "2023" which includes January 2024); same with widths. To highlight potential sexual dimorphism, the same method was used on all males versus all females. Then, the average length and width along the monitoring were tracked for each group (i.e. males, females, or juveniles) to highlight any potential life cycle-related variation. Finally, linear regressions were realized on the three groups, both years separately, to establish the relation between length and width, as well as to check if that relation remained steady from 2022 to 2023. All analyses described herein above were performed using RStudio 2023.12.1+402 (Rstudio Team 2020), alpha = 5%.

To serve as a proxy for the quantity of threats that *A. hilgendorfi* encounters in its environment, the number of locomotive legs of each specimen were also monitored, as well as the position of potential autotomized or missing legs. Several tests were performed to determine if the position of the lost legs was randomly distributed. For each month, the number of times each leg was missing was first compared with each other in a repeated-measure Friedman test. Then, Wilcoxon matched-pairs signed rank tests were realized after grouping left legs and right legs together; as well as front and back legs together. All statistical analyses performed here used GraphPad Prism 5.03 for Windows (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com), alpha = 5%.

Larval development

In June and July 2023, hydroids identified as *Obelia geniculata* (Linnaeus, 1758) and *Lafoea dumosa* (Fleming, 1820) based on Hayward and Ryland (1995) were collected on the hydrocaulus of bigger hydroids *Ectopleura larynx* (Ellis & Solander, 1786) (Figure 1G). They were preserved in 70% ethanol, and observed under a Keyence VHX-970F digital microscope. Larvae were counted and identified to the instar based on similarities with other *Ammothea* larvae (Cano-Sánchez and López-González 2013) and general information on protonymphon larvae development (Brenneis et al. 2017) – mainly, the number of developed walking legs and their number of segments were considered. Afterwards, they underwent chemical drying using hexamethyldisilazane (HMDS) (Bray et al. 1993). They were metalized using a Jeol JFC-1100E, and observed under a JSM-7200F Jeol Scanning Electron Microscope (SEM). The ratio of all instars was compared between June and July 2023. Some egg sacs were isolated from *A. hilgendorfi* males,

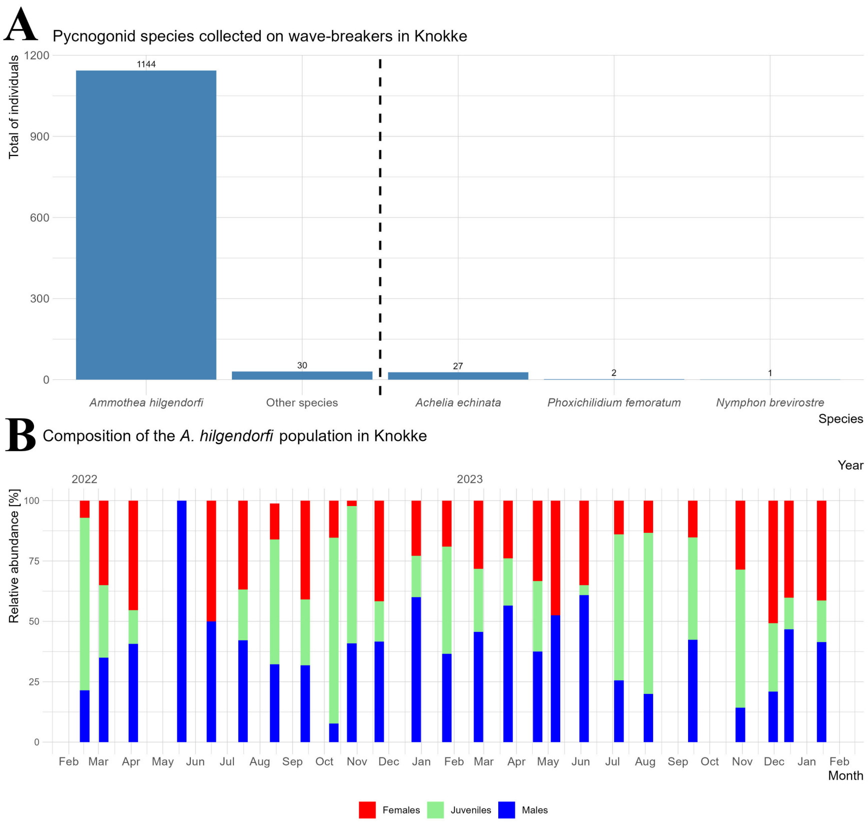

Figure 2. Sea spider population on the wave breakers of Knokke along the monitoring. A: Total number of specimens collected per species; B: Proportion of males (blue), females (red), and juveniles (green) *A. hilgendorfi* [%].

and pulled into multiwell plates filled with seawater. They were hatched as protonymphon larvae, then underwent the same protocol as above to be compared with the protonymphons collected directly on hydroids. The two earliest juvenile stages were also considered, most of them being collected on oysters alongside adults (sometimes even on the adult body). The goals were to demonstrate that the larvae present in Knokke belonged to *A. hilgendorfi*, and to track their development directly associated with their hosts.

Results

Population dynamics

A total of 1174 pycnogonid individuals were counted in 25 collection events from February 2022 to January 2024, among which 1144 *A. hilgendorfi* (97.4% of the total pycnogonids) (Figure 2A), for which the ratio of males, females and juveniles varied from one month to the other (Figure 2B). Among the other species collected, 27 were identified as *Achelia echinata* (Hodge, 1864) (2.3%), two as *P. femoratum* (0.2%) and one as a juvenile *N. brevirostre* (0.1%).

The population density of *A. hilgendorfi* ranged from 9 to 572 ind/m^2 with a mean of 206 ± 178 ind/m^2 (Figure 3A). The population showed acute

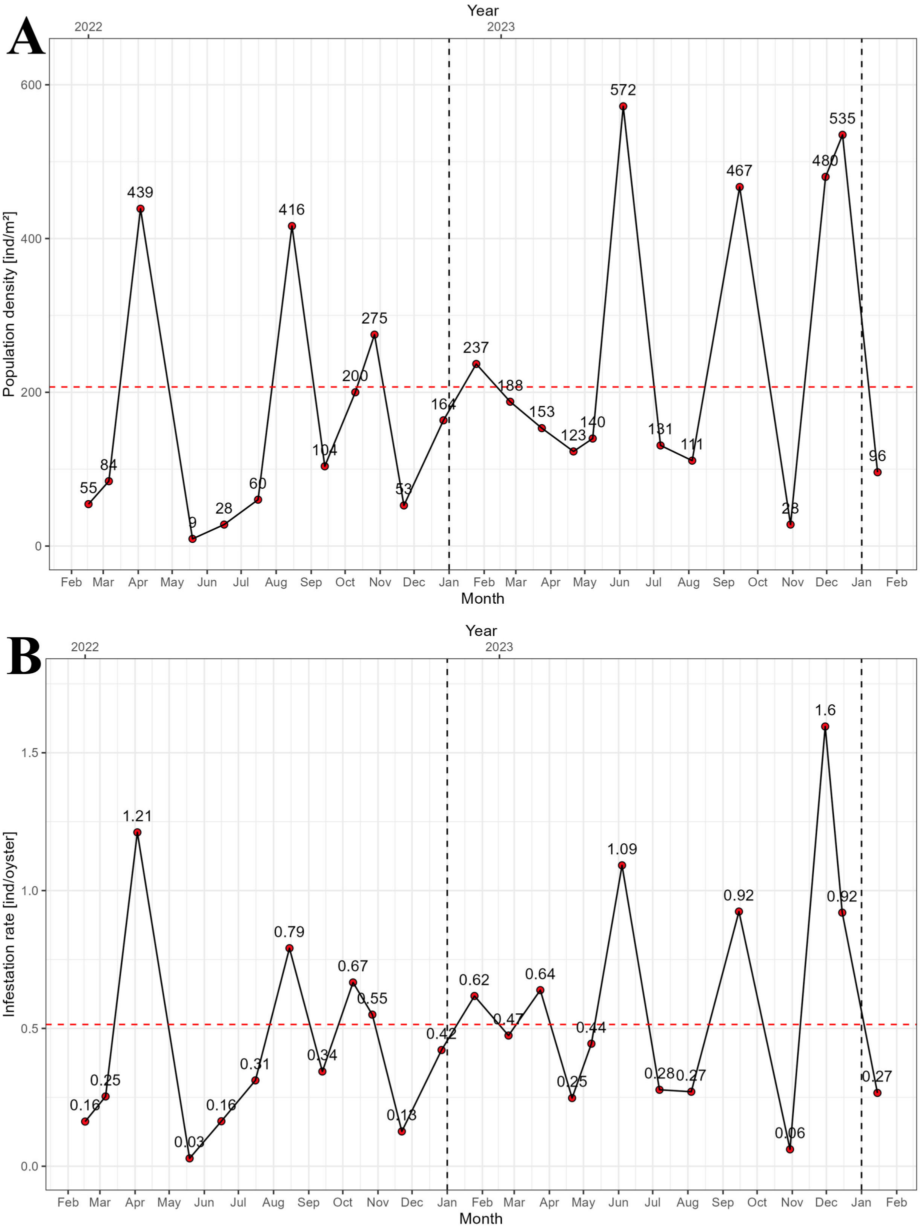

Figure 3. Variation of the abundance of *Ammothea hilgendorfi* along the monitoring. A: Density [ind/m²]; B: Infestation rate [ind/oyster]. Red dotted line: average during the entire monitoring.

variations from one month to another, characterized by high peaks in April 2022 (439 ind/m^2), August 2022 (416 ind/m^2), June 2023 (572 ind/m^2), September 2023 (467 ind/m^2) and in December 2023 (535 ind/m^2). On the other hand, low density occurred such as in May 2022 (9 ind/m^2) or October 2023 (28 ind/m^2). The density was fluctuating for most of the monitoring as it suddenly went from a high maximum to a low minimum and/or vice versa several times. Despite that apparent instability, an increasing tendency was noticed from 2022 to 2023. The maxima and minima were generally higher in 2023 than in 2022 as well as the mean population density, 153 ± 154 ind/m^2 in 2022 and 264 ± 192 ind/m^2 in 2023. Similarly, among the 1144 *A. hilgendorfi* specimens collected, only 425 specimens were collected in 2022 against 719 in 2023, although comparable collecting efforts were provided (i.e. total sampling area of 2.49 m^2 in 2022 and 3.02 m^2 in 2023). That represents 171 specimens per m^2 in 2022 and 239 specimens per m^2 in 2023, an increase of 140%. Also, the period of the minima and maxima did not match from one year to the other (i.e. no apparent seasonality).

The oyster infestation rate, or number of pycnogonids per oyster, generally followed a tendency similar to the density (Figure 3B). It reached maxima in April 2022 (1.21), June 2023 (1.09) and November 2023 (1.60, absolute maximum). Infestation coefficients > 1 mean that more *A. hilgendorfi* were present in our sample than oysters. Over the whole monitoring period, the infestation coefficient ranged from 0.03 to 1.60 with an average of 0.51 ± 0.40. Therefore, it could be expected to find on average half the number of *A. hilgendorfi* than the number of oysters.

Within the *A. hilgendorfi* population, 28.8% were juveniles (330 ind), 30.7% females (351 ind), and 40.5% males (463 ind), 36.9% of which were bearing eggs (171 ind). Yet, the ratios were not constant over time (Figure 4). The percentage of ovigerous males shows a yearly periodicity (Figure 4C). A first peak was observed beginning in April and ending in July in 2022; a second one from April to June 2023. However, ovigerous males were also observed in low numbers during the entire monitoring. The juvenile proportion (Figure 4A) followed a similar pattern with a time lag of ~ 4 months. A first juvenile peak started in August until November 2022; a second one ranged from July to November 2023. Consequently, the end of the ovigerous male peaks matched the start of the juvenile peaks. The sex ratio, however, lacked periodicity (Figure 4B), usually ranging from 1:1 to 2:1, occasionally up to 9:1 (October 2022). Although males generally outnumbered females, they were in significant majority only six times. Rarely, more females were collected than males but only once was that difference significant (November 2023).

Biometric measurements

Table 2 summarizes the length and width of *A. hilgendorfi* (excluding 15 unmeasured specimens in 2023). Despite each group being slightly smaller the second year in almost all metrics, a significant difference between 2022 and 2023 was only found in the average female length ($p = 9*10^{-4}$). All metrics were significantly different between males and females from the same year ($p = 2*10^{-3}$ and 10^{-2} for the lengths of 2022 and 2023 respectively; and $5.767*10^{-5}$ and $< 2.2*10^{-16}$ for the widths).

The average lengths and widths varied along the monitoring, the size of the confidence interval (CI) depending on the number of individuals collected (Figure 5). From November to December (plus January in the second year), adults tended to be smaller. This matches with the juvenile peaks, which ended in November in both years. The period with smaller juveniles ranged both years from July to August. That also matches the end of the ovigerous male peaks, as well as the start of the juvenile peaks, particularly visible in the second year.

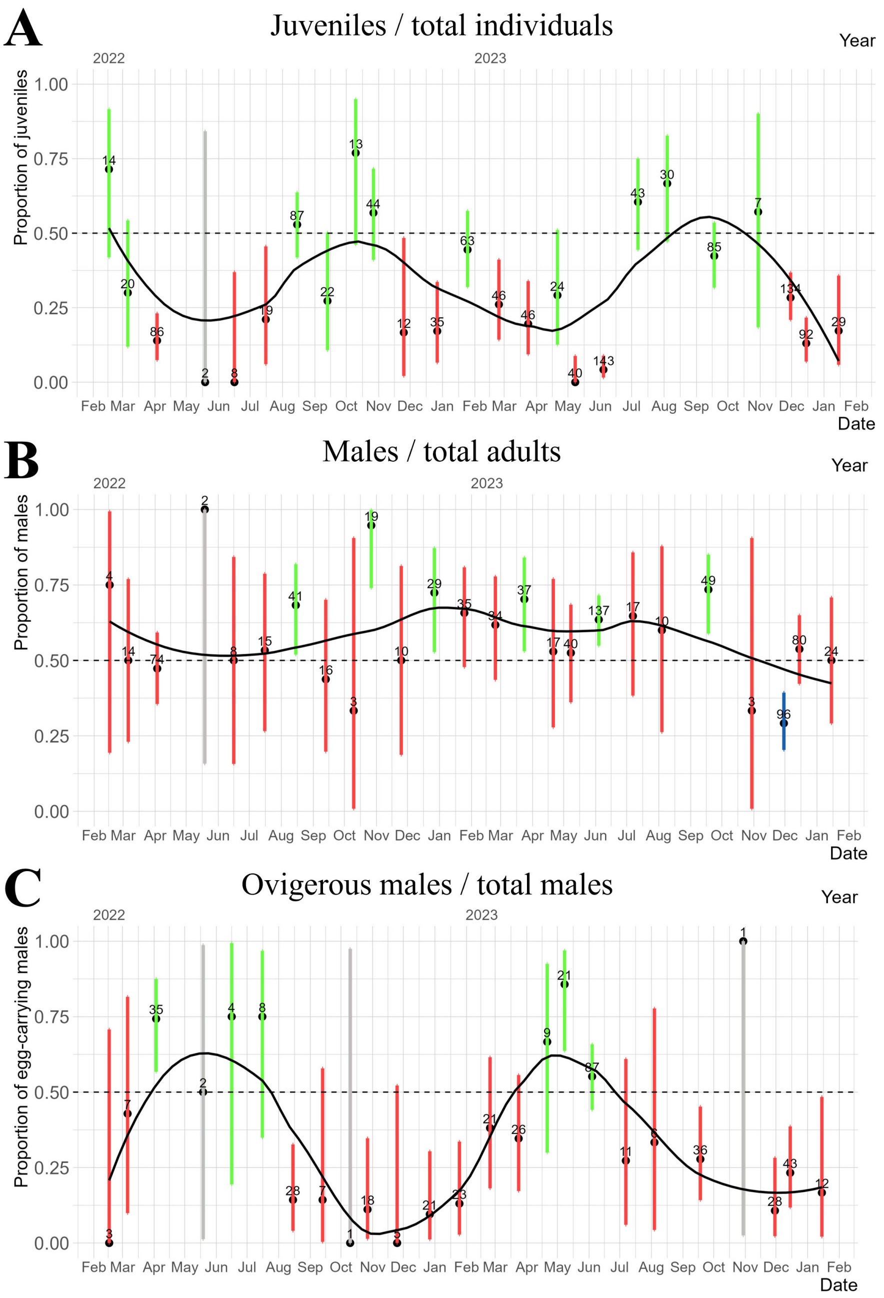

Figure 4. Composition of the population of *Ammothea hilgendorfi* along the monitoring. Black curves created by LOESS local regression (span: 0.5) A: Ratio of juveniles in the population to the total number of individuals. The number of individuals collected is displayed for each month. Green error bars: CI95 include 0.5; red error bars: CI95 completely below 0.5; gray error bar: less than 3 *A. hilgendorfi* collected (month not considered). B: Adult sex ratio; ratio of males to the total number of adults. The number of adults collected is displayed each month. Green error bars: CI95 completely above 0.5 (significantly more males than females); red error bars: CI95 include 0.5 (sex ratio roughly balanced); blue error bar: CI95 completely below 0.5 (significantly more females than males); gray error bar: less than 3 adults collected (month not considered). C: Ratio of ovigerous males to the total number of males. The number of males collected is displayed each month. Green error bars: CI95 in majority above 0.5; red error bars: CI95 in majority below 0.5; gray error bars: less than 3 males collected (month not considered).

The relation between length and width for each group in both years are shown in Table 3 (see Figure S1 for associated graphs). The regression lines remained steady from one year to the other in every group; only slight variations were observed.

Table 2. Biometric measurements of *Ammothea hilgendorfi*, mean ± standard deviation of length and width [mm] of males, females, and juveniles in 2022 and 2023. * indicates significant differences between 2022 and 2023.

		2022	2023
Males (N = 162 and 298)	Length Width	2.0 ± 0.2 1.5 ± 0.2	2.0 ± 0.2 1.6 ± 0.2
Females (N = 108 and 238)	Length* Width	2.2 ± 0.2 1.4 ± 0.2	2.1 ± 0.2 1.4 ± 0.2
Juveniles (N = 155 and 168)	Length Width	1.4 ± 0.3 0.9 ± 0.2	1.3 ± 0.3 0.9 ± 0.2

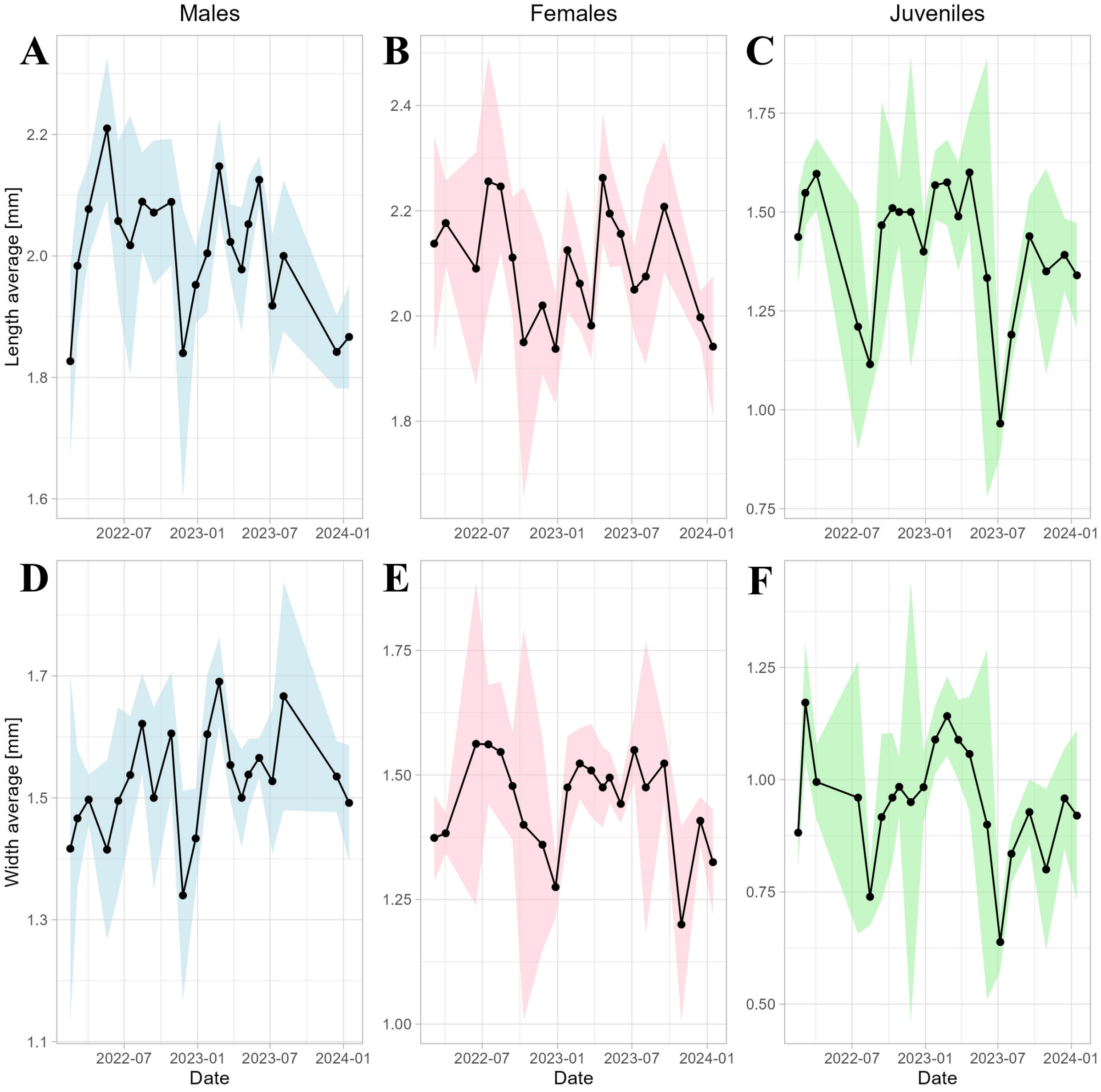

Figure 5. Evolution of the average width and length of *Ammothea hilgendorfi* along the years 2022 and 2023 [mm]. A, B, C: Average length respectively for males, females and juveniles. D, E, F: Average width respectively for males, females and juveniles of *A. hilgendorfi*. Only months in which N > 3 were considered. Colored surfaces represent the CI95.

Among the 1129 measured *A. hilgendorfi*, 694 had all their eight legs (61.5%), 263 had seven (23.3%), 110 had six (9.7%), 38 had five (3.4%), 17 had four (1.5%), 6 had three (0.5%), and 1 individual had only two legs (0.1%).

Table 3. Relation between width (W) and length (L) for males, females, and juveniles of *Ammothea hilgendorfi* during both years of the monitoring.

	2022	2023
Males	W = 0.5 + 0.5 L	W = 0.4 + 0.6 L
Females	W = 0.6 + 0.4 L	W = 0.5 + 0.5 L
Juveniles	W = 0 + 0.6 L	W = 0 + 0.7 L

Table 4. Number of times each walking leg was missing in *Ammothea hilgendorfi.*

	Left	Right
1st	90 (12.8%)	119 (17.0%)
2nd	86 (12.3%)	86 (12.3%)
3rd	82 (11.7%)	83 (11.8%)
4th	81 (11.6%)	74 (10.5%)

Table 5. Relative abundance of larval stages collected on hydroids in Knokke.

Month	Protonymphon	Postlarva 1	Postlarva 2	Postlarva 3	Postlarva 4	Juvenile 1	Juvenile 2
June	20 (56%)	6 (17%)	4 (11%)	2 (5%)	4 (11%)	0 (0%)	0 (0%)
July	2 (67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)

The total number of missing legs in the entire pool was thus 701 legs. Table 4 summarizes the number of times a given leg was missing. The Friedman test revealed no significant difference by comparing every leg to each other ($p = 0.10$). There was neither between left and right legs ($p = 0.27$), but there was between front and back legs ($p = 0.049$). Front legs were then slightly more often absent than back legs.

Larval development

All larval instars, even early juveniles, were observed parasitizing hydrants of *O. geniculata* and *L. dumosa* but none were present directly on the larger *E. larynx* (Figure 1G). They were always found outside their host (i.e. ectoparasites). The number of each instar collected in both months is accessible in Table 5. Living protonymphon larvae hatched after a few days in multiwell plates. They could survive for over a week without having access to a host to parasitize.

Based on Figure 6A–C, the *A. hilgendorfi* protonymphon larvae hatch from the eggs attached to the male ovigers and remain aggregated together. Then, they infest a hydrozoan which becomes their host for the rest of the larval development. The observed protonymphons, both hatching from egg sacs and the ones collected in the field, are characterized by a small size, a round shape, a proboscis, three pairs of appendages and no walking legs (Figures 6D, S2), which is very similar to other *Ammothea* spp. protonymphons (Cano-Sánchez and López-González 2013). Eye pigments are visible but at this stage, the eye bulb is not developed (Figure S2C–E).

The first pair of appendages are the chelifores, located anteriorly, longer than the proboscis and about the same length as the body. They are made of two articles: the scape, bearing the spinning spine that helps the protonymphon to anchor itself on the hydrozoan (Burris 2011) (Figures 6B, F, S2A); and the chelae, ending in a claw made of two opposed articles: a mobile

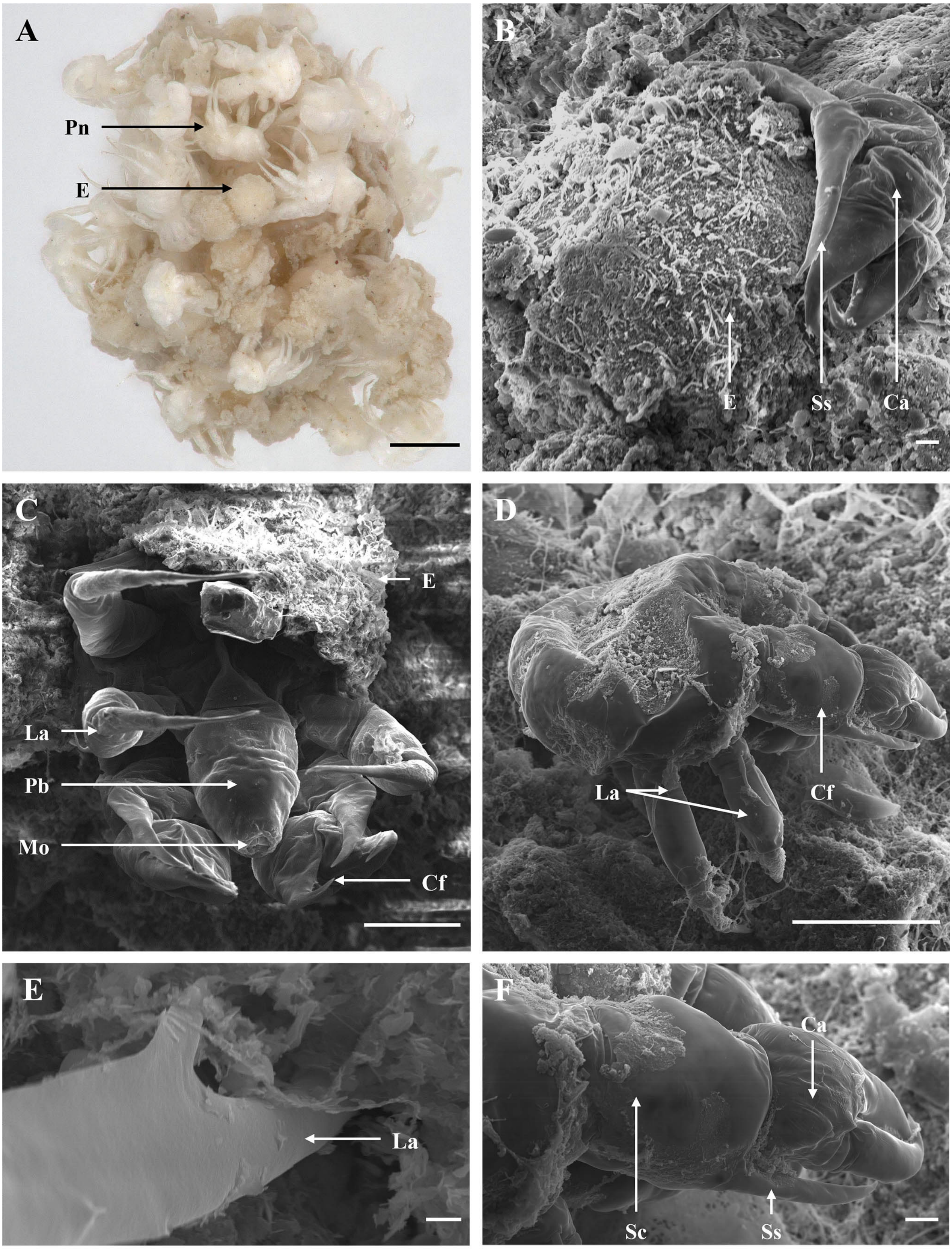

Figure 6. Hatching eggs and protonymphon larvae of *Ammothea hilgendorfi*. A: Egg sac with hatching protonymphons, chemically dried; B–F: Larvae under Scanning Electron Microscopy (SEM); B: Chelifore of a protonymphon getting out of his egg; C: Hatching protonymphon, ventral view; D: Protonymphon on a hydrozoan, rear view; E: End of the larval appendage last article, rear view; F: Chelifore of a protonymphon, rear view. Pn: Protonymphon; E: Egg; Pb: Proboscis; Mo: Mouth; Ss: Spinning spine; Ca: Chela; La: Larval appendage; Cf: Chelifores; Sc: Scape. Scale bars: A: 200 µm; B: 10 µm; C–D: 100 µm; E: 10 µm; F: 1 µm. Photographs ©Benjamin Decoux.

finger and an opposed fixed finger, both indented (Figures 6F, S2B, F). The chelae also help to grasp the host. There are two other appendages ventrally, the larval appendages I and II (LA I and LA II). Both are of the same size and consist of three segments, the last of which has unarticulated pliers shaped ends (Figure 6E). This shows that the larvae are fully equipped to parasitize and hold on to hydrozoans from the very first stage. The presence of a spinning spine and complete larval appendages are known in the literature to be characteristic of an ectoparasitic lifestyle (Burris 2011).

The first postlarval instar (i.e. the second stage of post-embryonic development) is comparable to the previous stage. The main distinctions are the emergence of the first walking leg primordia (Figure 7A–D), in juxtaposition to the newly formed abdomen (Figure 7E). The larva remains attached to the hydrozoan by the chelifores and the spinning spines (Figure 7F), with the emerging legs oriented upwards. The eye pigments appear a bit more opaque than the last stage (Figure 7A, B).

In the second postlarval instar, the first walking leg is segmented but not complete, exhibiting six articles instead of eight (excluding the claw) in a fully developed leg (Figure 8A–C). Starting basally, an incomplete leg is composed of three coxae, followed by the fused femur-tibia I, tibia II and the fused tarsus-propodus (Cano-Sánchez and López-González 2013), which ends with three claws: the main claw and two smaller auxiliary claws, approximately half the size of the main claw in this species (Figure 8E). Similarly to the second instar, the primordia of the second pair of walking legs emerges juxtaposed to the abdomen (Figure 8A–D). At this point in the development, the larva remains attached to the hydrozoan using its chelifore, with the spinning spine (Figure 8F), and larval appendages. The eye bulb has not yet formed, but the eye pigments are more visible and start to be subdivided into four distinct parts (Figure 8C).

In the third instar, the eye bulb is visible (Figure 9A–C), the first walking leg is completed (i.e. eight articles) and the second walking leg has developed six segments. The primordia of the third walking leg are juxtaposed to the abdomen. The spinning spines and larval limbs are still visible (Figure 9D). The larva is now also able to stand horizontally on the hydrozoan, grasping it with its legs (Figure 9E).

The fourth and final postlarval instar starts to clearly resemble juveniles of *A. hilgendorfi*. Indeed, this instar is equipped with two pairs of fully developed walking legs and two pairs of incomplete legs (Figure 10A–D, F). The third walking leg is indeed composed of only six articles and only the primordia of the fourth pair of legs is present. The eye bulb is now a discernible dome with two pairs of eyes close to each other, and the spinning spines and larval limbs remain visible (Figure 10E).

The earliest juvenile stage of *A. hilgendorfi* is characterized by the development of the last pairs of walking legs to six articles (Figure 11A, D). LAs I are substituted by a pair of 3-segmented palps (Figure 11B), the chelifores

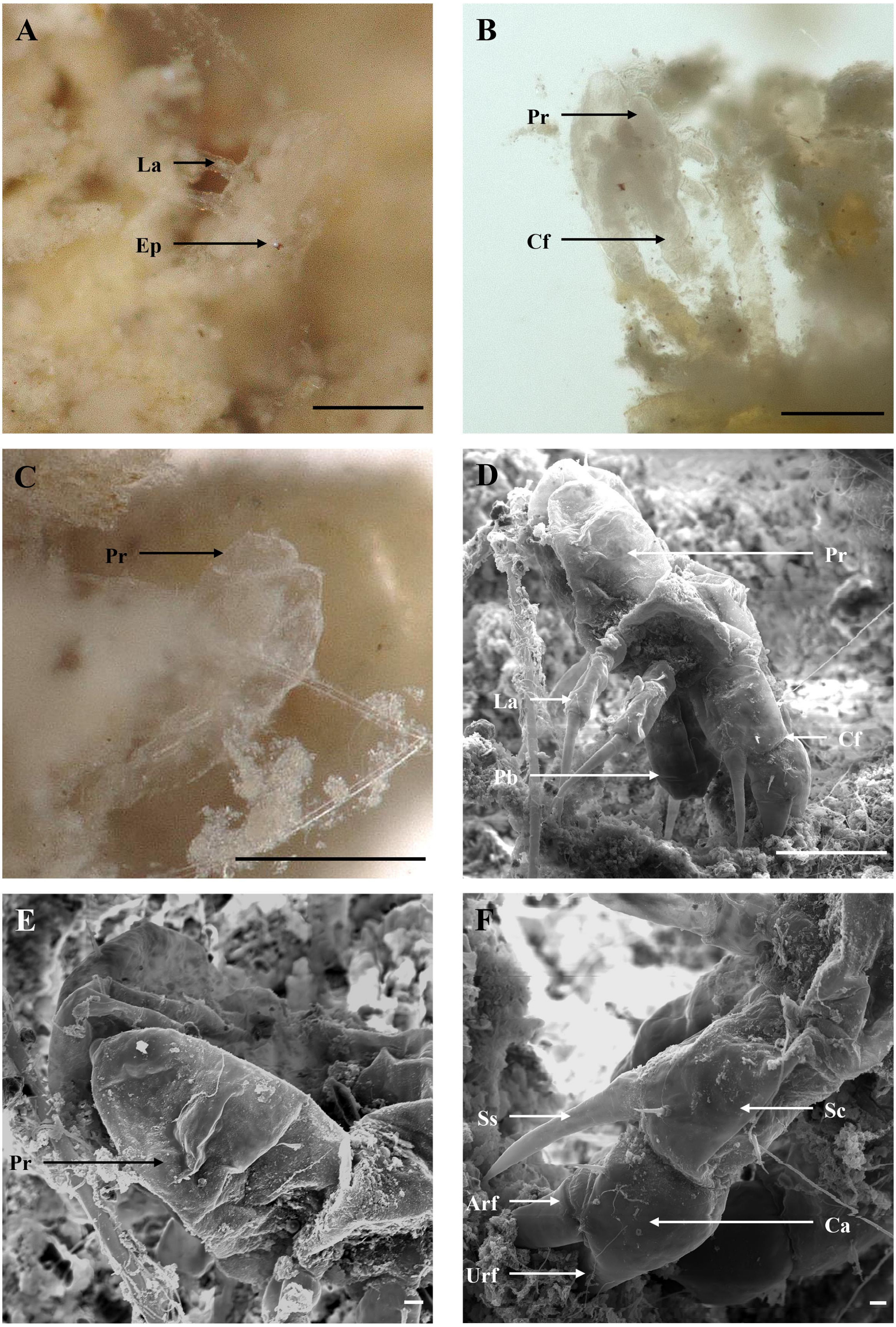

Figure 7. First postlarval instar of *Ammothea hilgendorfi* on hydroid hosts. A–C: First postlarval instar in 70% ethanol; A: Profile view; B: Top view; C: Rear view; D–F: First postlarval instar under SEM D: Profile view; E: Primordia of the first postlarval instar, top view; F: Chelifore of a first postlarval instar, Profile view; La: Larval appendage; Ep: eye pigments; Pr: Primordia; Cf: Chelifores; Pb: Proboscis; Ss: Spinning spine; Sc: Scape; Arf: Articulated finger; Ca: Chela; Urf: Unarticulated finger; Scale bar: A–B: 200 µm; C: 250 µm; D: 100 µm; E–F: 10 µm. Photographs ©Benjamin Decoux.

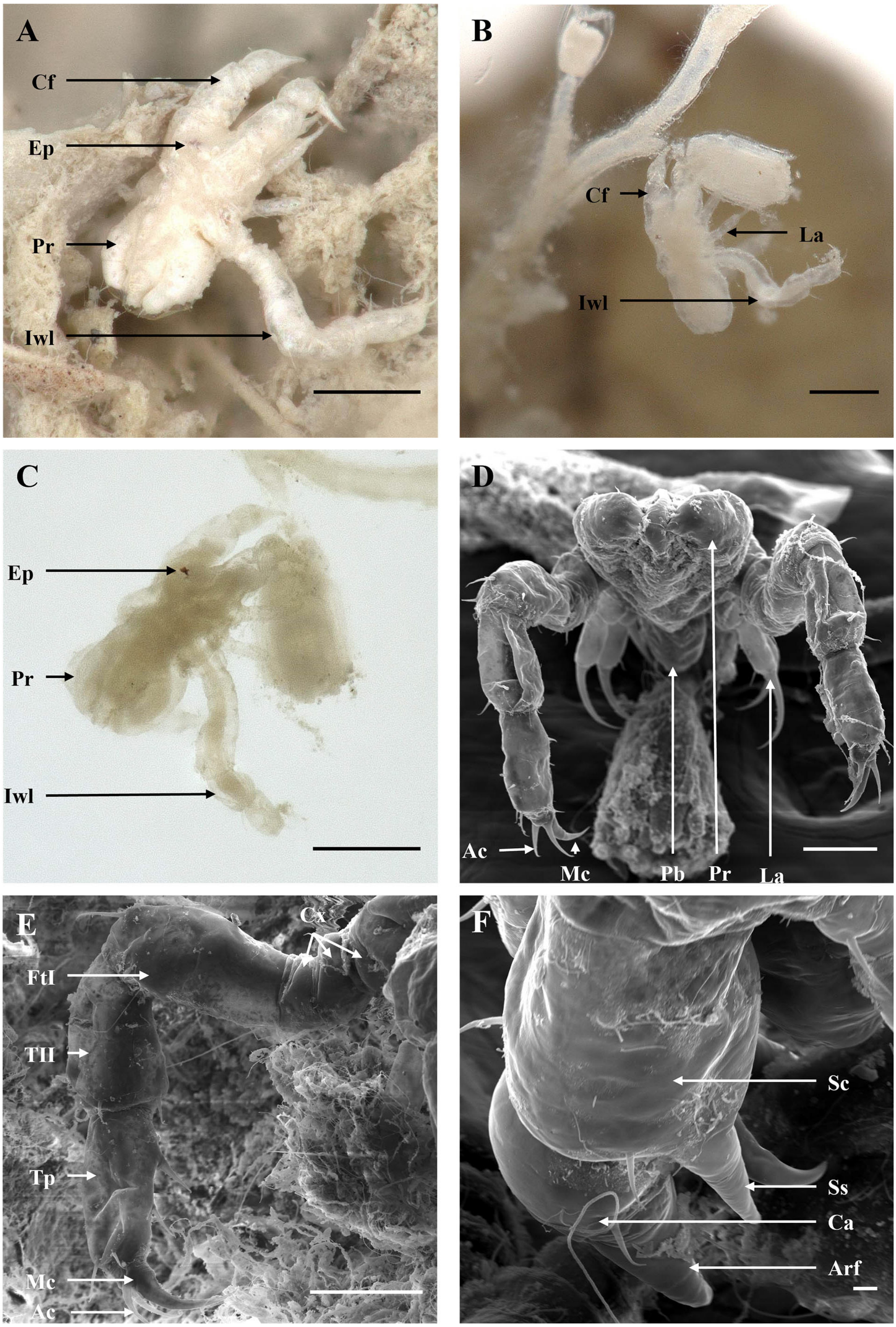

Figure 8. Second postlarval instar of *Ammothea hilgendorfi* on hydroids hosts. A: Postlarval instar chemically dried, rear view; B–C: Postlarval instar in 70% ethanol; B: Profile view; C: Rear view; D–F: Second postlarval instar under SEM; D: Rear view; E: Incomplete first walking leg of a second postlarval instar, front view; F: Chelifore of a second postlarval instar, rear view; Cf: Chelifores; Ep: eye pigments; Pr: Primordia; Iwl: Incomplete walking leg; La: Larval appendage; Ac: Auxiliary claw; Mc: Main claw; Pb: Proboscis; Cx: coxa; FtI: Femur-tibia I; TII: Tibia II; Tp: Tarsus-propodus; Arf: Articulated finger; Sc: Scape; Ss: Spinning spine; Ca: Chela; Scale bar: A: 200 µm; B–C: 250 µm; D–E: 100 µm; F: 10 µm. Photographs ©Benjamin Decoux.

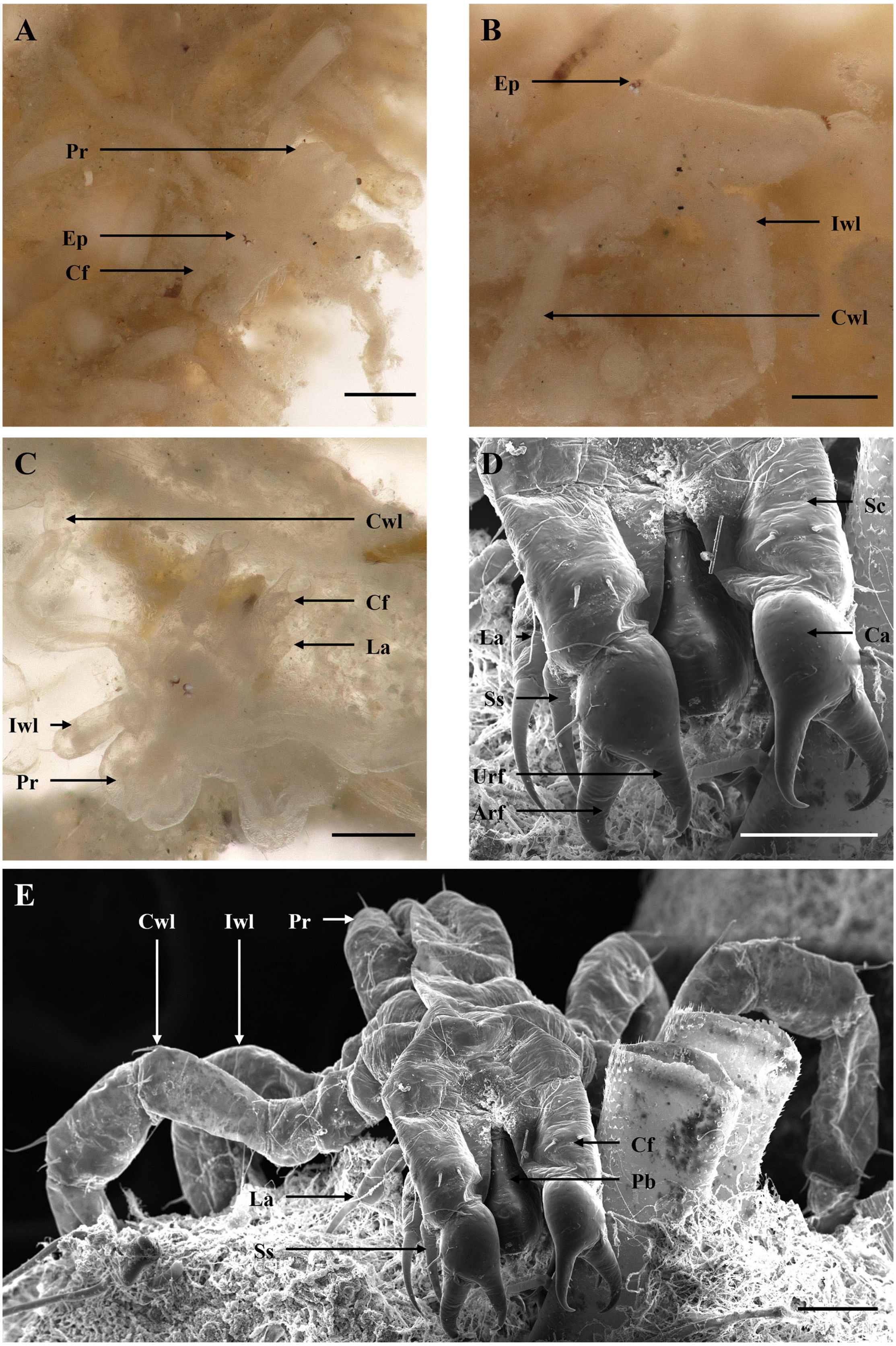

Figure 9. Third postlarval instar of *Ammothea hilgendorfi* on hydroids hosts; A–C: Postlarval instar in 70% ethanol; A: Top view; B: Profile view; C: Front view; D–E: Third postlarval instar under SEM; D: Chelifores of the third postlarval instar, front view; E: Third postlarval instar standing on a hydrozoan horizontally, front view; Pr: Primordia; Ep: Eye pigment; Cf: Chelifores; Iwl: Incomplete walking leg; Cwl: Complete walking leg; La: Larval appendage; Sc: Scape; Ca: Chela; Ss: Spinning spine; Urf: Unarticulated finger; Arf: Articulated finger; Pb: Proboscis; Scale bar: A: 250 µm; B–C: 200 µm; D–E: 100 µm. Photographs ©Benjamin Decoux.

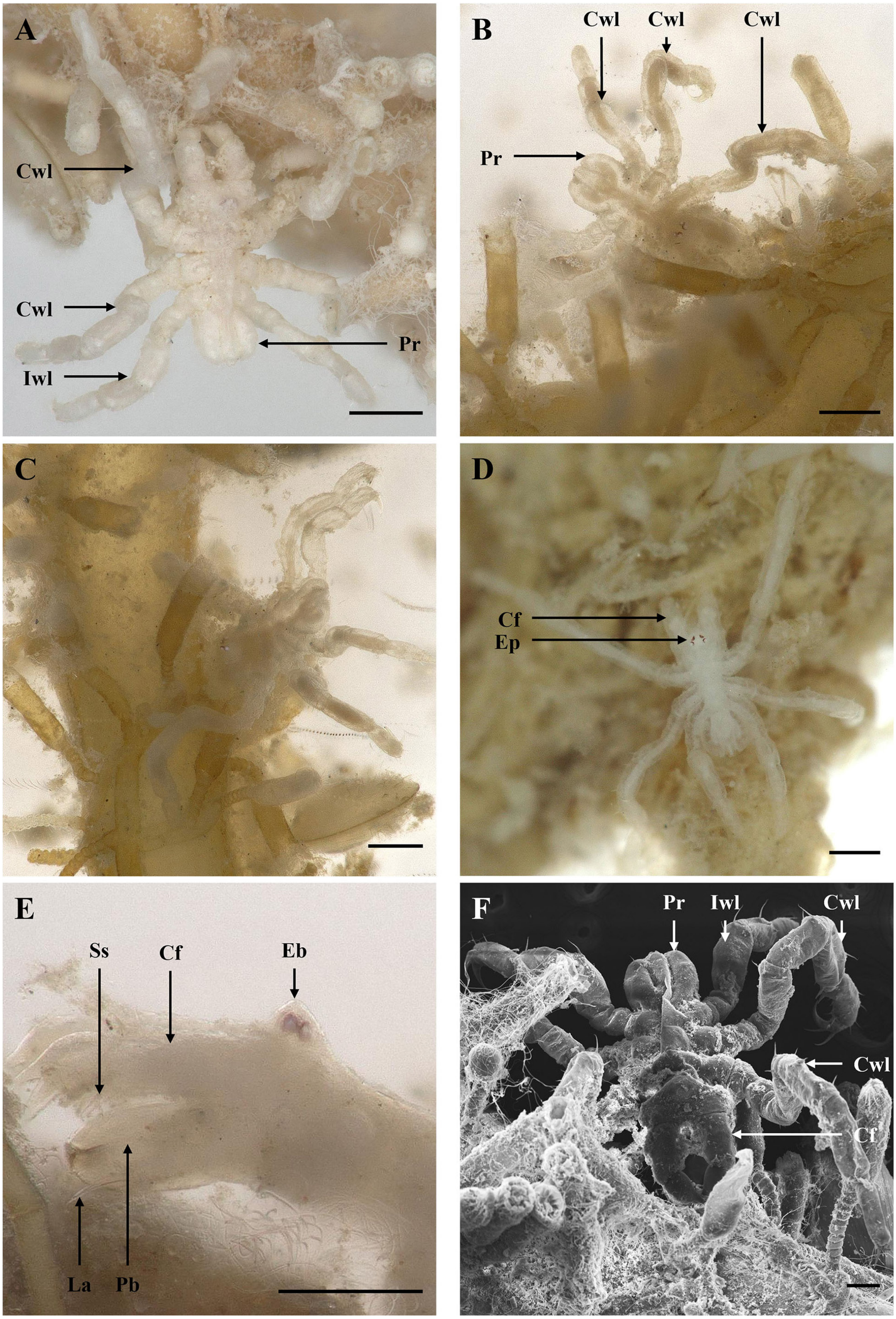

Figure 10. Fourth postlarval instar of *Ammothea hilgendorfi* on hydroids hosts; A: Chemically dried postlarval instar, top view; B–E: Postlarval instar in 70% ethanol; B: Front view C: Rear view; D: Top view; E: Anterior part of the fourth postlarval instar; profile view; F: Fourth postlarval instar under SEM; front view; Cwl: Complete walking leg; Iwl: Incomplete walking le g; Pr: Primordia; Cf: Chelifores; Ep: Eye pigment; Ss: Spinning spine; Eb: Eye bulb; La: Larval appendage; Pb: Proboscis; Scale bar: A–D: 250 µm; E: 200 µm; F: 100 µm. Photographs ©Benjamin Decoux.

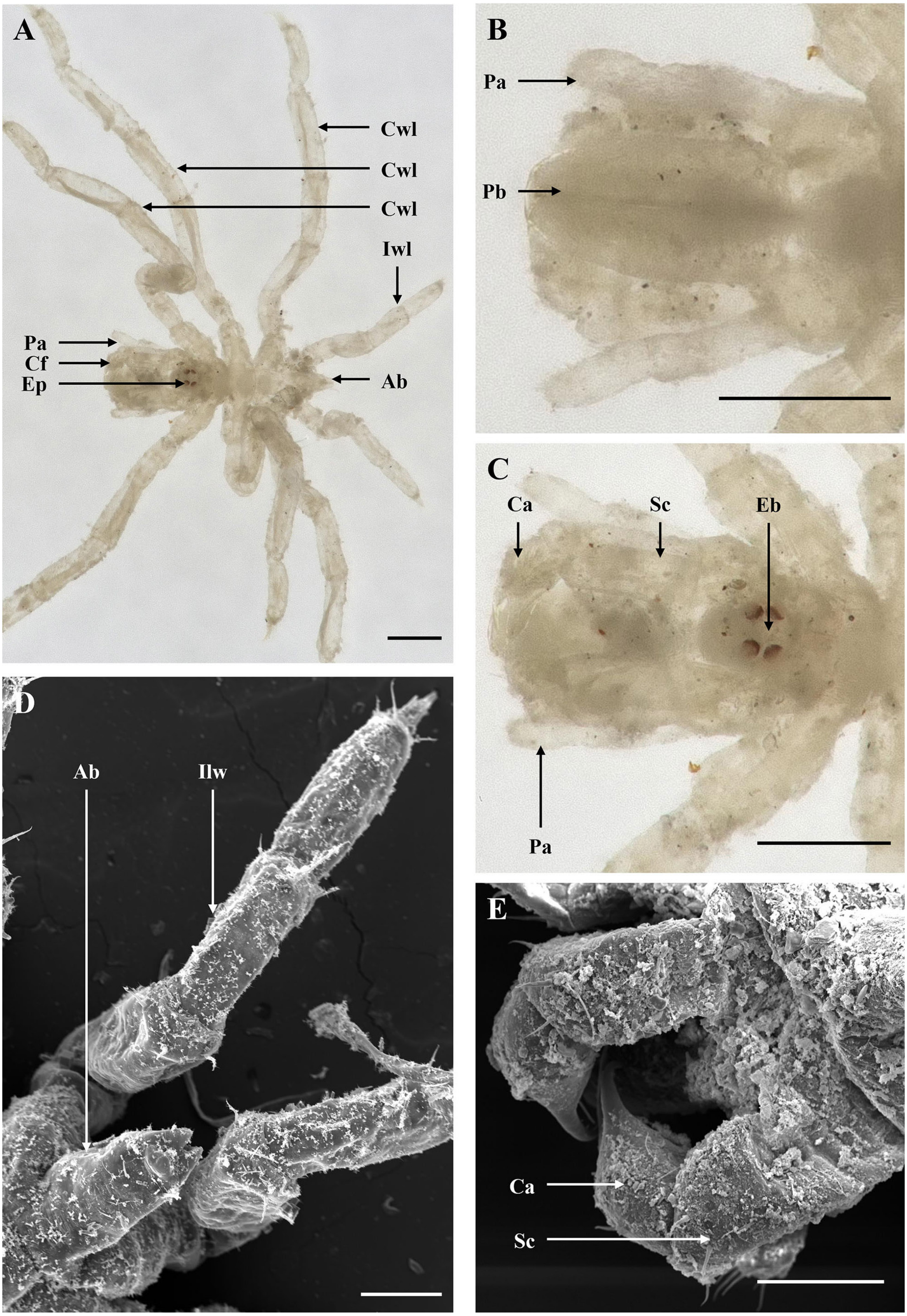

Figure 11. First juvenile stage of *Ammothea hilgendorfi*; A–C: First juvenile in 70% ethanol; A: Top view; B–C: Anterior part of the first juvenile; B: Bottom view; C: Top view; D–E: First juvenile stage under SEM; D: Fourth walking leg, top view E: Chelifores, top view; Cwl: Complete walking leg; Iwl: Incomplete walking leg; Pa: Palp; Cf: Chelifores; Ep: Eye pigment; Ab: Abdomen; Pb: Proboscis; Ca: Chela; Sc: Scape; Eb: Eye bulb; Scale bar: A: 250 µm; B–C: 200 µm; D–E: 100 µm. Photographs ©Benjamin Decoux.

are still present (Figure 11C, E) and the eye bulb is well formed with four distinct eyes (Figure 11C). Therefore, the first juvenile exhibits morphological traits of both a postlarval instar (i.e. incomplete walking legs) and an adult (i.e. palps).

The second juvenile stage grows a fully developed last pair of walking legs (Figure 12A, D). Like adults, their palps are now 9-articulated (Figure 12C), but unlike adults, they still possess two-segmented chelifores (i.e. scape and chelae) (Figure 12B, E).

Discussion

Density and abundance

Densities as high as 206 ± 178 ind/m^2, with a maximum of 572 ind/m^2 (Figure 3), are among the greatest densities observed for some native sea spiders: almost 800 ind/m^2 in *P. litorale* (Wilhelm et al. 1997), 713 ind/m^2 in *Anoplodactylus pygmaeus* (Hodge, 1864), 175 ind/m^2 in *A. echinata*, 150 ind/m^2 in *Ammothella appendiculata* (Dohrn, 1881), 631 and 3656 ind/m^2 in *Tanystylum conirostre* (Dohrn, 1881) (Perez-Ruzafa and Munilla 1992), and 1900 ind/m^2 in *Phoxichilidium tubulariae* (Lebour, 1945) (now accepted as *P. femoratum*) (Lovely 2005). Although some species may sporadically form dense populations, the expected sea spider densities are usually much lower: 71 ind/m^2 in *P. litorale* (Schmidt and Bückmann 1971), most records lower than 25 ind/m^2 in the Mar Menor (Murcia, Spain) (Perez-Ruzafa and Munilla 1992), 40 ind/m^2 in *Nymphon hirtipes* (Bell, 1855) (Arnaud and Bamber 1988), and on average 5 ind/m^2, maximum 14 ind/m^2, in *Tanystylum cavidorsum* (Clark, 1972) (now accepted as *T. antipodum* (Clark, 1977)) (Blankley and Grindley 1985). Sabroux et al. (2019) collected approximately 800 pycnogonids in the Martinique Islands but required huge collecting efforts (> 500 sampling events and 45 people involved). Abyssal species are even scarcer: from 21 to 7472 ind/ha considering all pycnogonids in Laubier et al. (1976), and 0.87 ind/ha in *Colossendeis colossea* (Wilson, 1881) (Grassle et al. 1975). Therefore, the present population of *A. hilgendorfi* can be regarded as one of the densest populations of sea spiders in the world, and likely the densest of *A. hilgendorfi* recorded to this day. Indeed, the number of specimens collected elsewhere in Europe was very limited (Table 1). Still, in other place were reported high densities: in the Netherlands (Faasse 2013), in England (Bamber 2012), and 3.5 ind/m^2 (70 specimens in 20 m^2) in France (Le Roux et al. 2020), with punctual gathering at up to 15 specimens within 1 dm^2 (Le Roux et al. 2022). It must also be noted that the present collecting method, which was indirect, only considered a surface of oysters. It did not include the rocks underneath, neither the potential individuals that could be found in the subtidal area, which would have been impossible due to the way the rocks were assembled in the wave breakers and the high turbidity of water. Hence, the recorded densities are most likely underestimated. This is why the infestation

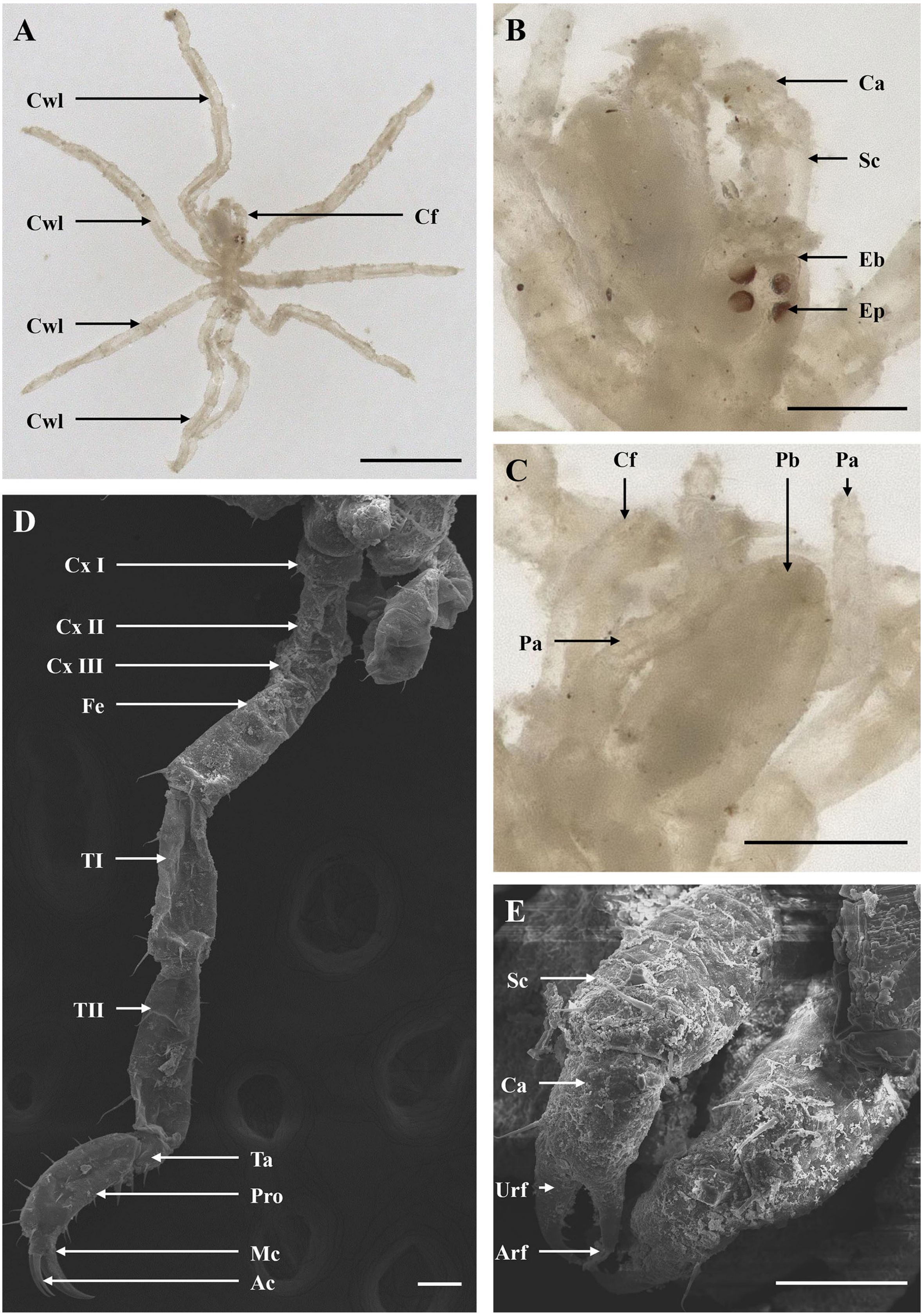

Figure 12. Second juvenile stage of *Ammothea hilgendorfi*; A–C: Second juvenile in 70% ethanol; A: Top view; B–C: Anterior part of the second juvenile; B: Top view; C: Bottom view; D–E: Second juvenile stage under SEM; D: Fourth walking leg, top view E: Chelifores, top view; Cwl: Complete walking leg; Cf: Chelifores; Ca: Chela; Sc: Scape; Eb: Eye bulb; Ep: Eye pigment; Ss: Spinning spine; Pb: Proboscis; Cx: Coxa; Fe: Femur; TI: Tibia I; TII: Tibia II; Ta: Tarsus; Pro: Propodus; Mc: Main claw; Ac: Auxiliary claw; Urf: Unarticulated finger; Arf: Articulated finger; Scale bar: A: 1000 µm; B: 200 µm; C: 250 µm; D–E: 100 µm. Photographs ©Benjamin Decoux.

rate of *A. hilgendorfi* on *M. gigas* was measured, as another way to compare their abundance with other populations, which might be easier depending on the substrate.

The density of the present population was quite unstable from one month to another (Figure 3). This might be inherent to sea spiders: Wilhelm et al. (1997) and Lovely (2005) also observed fluctuations in respectively *P. litorale* and *P. tubulariae*. Events of collapsing and exploding densities in populations are referred to as boom-and-bust and are known to happen in alien species invasions, especially recently established ones (Blackburn et al. 2011; Strayer et al. 2017), which might be the case for *A. hilgendorfi* in Belgium. While such population crashes can lead to local extinction, thereby being a one-time event, they may appear as rather recurrent and repeated, following or not a cyclic tendency (Strayer et al. 2017). Boom-and-bust events can also progressively disappear or temporarily fade among populations (Simberloff and Gibbons 2004; Strayer et al. 2017). Such phenomena could thus, at least partly, be involved in the observed density fluctuations. However, the putative boom-and-bust episodes occurred with a high frequency and then might arise from other causes. For instance, periodic migrations have been shown in other pycnogonids: *N. gracile* operates seasonal migrations from shallow waters to offshore in winter (Fage 1932; Morgan 1978; Arnaud and Bamber 1988), and *P. tubulariae* from shallow to offshore waters according to reproduction periods (Lovely 2005). In the current study, samples were exclusively collected on oyster reefs. Thus, periodic migrations of *A. hilgendorfi* in deeper water might explain why its density on wave breakers varied that much, especially since it has already been punctually observed in the Hinderbank at 31 m deep and 30 km offshore from Belgian coasts (Faasse 2020, Flandroit and Faasse 2024). The measured lowest density interestingly matches the only rainy collecting event (Table S1), raising the question on how the weather could somehow trigger some migration, as it was shown to influence insect behavior and population fluctuations (Weisser et al. 1997). The variations could also be due to potential new arrivals of *A. hilgendorfi*, as there might remain a flow between several sub-populations of the same species (Slatkin 1985). Finally, such differences from one month to the other might simply be due to randomness. While some species follow a random spatial distribution, in which case the measured densities are expected to remain consistent (Stoffels et al. 2003), Le Roux et al. (2020) noted a tendency to local aggregation in *A. hilgendorfi*. If this species is indeed gregarious, the randomly selected surface will feature great density variations depending on where it was in the ecosystem (Stoffels et al. 2003). Further studies on *A. hilgendorfi* potential gregariousness should help assess this hypothesis.

The measured densities can also be extrapolated to the entire wave-breaker ecosystem. They are twenty, with a total measured surface reaching approximately 47660 m^2 (Google Maps 2024). This would mean that there were, on average, nearly 10 million *A. hilgendorfi* in the area during the survey. The actual number can be hypothesized as lower, considering that not all the wave-breaker surface is suitable (i.e. proximal areas with little biofouling and central areas without any). However, the actual number can also be regarded as higher, since there might be other suitable substrates in the region, similar to the Hinderbank (Faasse 2020; Flandroit and Faasse 2024). In both cases, the number of *A. hilgendorfi* present there is enormous.

Phenology

The great sex ratio imbalances observed in *A. hilgendorfi* (oftentimes in favor of males) could be explained by the exclusive paternal care of pycnogonids (Bain and Govedich 2004). Indeed, it was observed that in *N. hirtipes*, females died quickly after reproduction when they became "purposeless", whereas males lived for roughly nine months afterwards (Mercier et al. 2015). Similar phenomena could be at play in *A. hilgendorfi*. Barreto and Avise realized samplings of the species *A. hilgendorfi* (Barreto and Avise 2008) and *Pycnogonum stearnsi* Ives, 1883 (Barreto and Avise 2010). In *A. hilgendorfi*, 58% specimens out of 119 adults were males. In *P. stearnsi*, close to 50% of the 123 sampled adults were males (61/123). Excluding juveniles, 463/814 adults *A. hilgendorfi* were males (57%) in the present survey, which is very similar to Barreto and Avise (2008), hence it might be normal in this species and unrelated to their recent invasion history in Belgium.

The periodicity observed in the life cycle of *A. hilgendorfi* made it possible to estimate its duration at 7–8 months, from eggs to mature adults. Indeed, the reproduction peaks started in April, leading to high abundances of ovigerous males. The larva ratio could not be monitored for a yearlong because their actual host was uncertain at the time (see below). Still, it can be inferred that, after the hatching of the protonymphon larvae, they find a host to parasitize and, through successive molting, reach the free-living juvenile stage 3–4 months later (July–August; measured juvenile peaks). Juveniles then need an additional 4 months to become mature adults, as shown by the large proportion of adults in the November–December pool. In the spectrum of other sea spiders, 7–8 months is average to short: 21 days from hatching to juveniles in *P. femoratum* (Lovely 2005), 5 months from hatching to adults in *Propallene longiceps* (Bohm, 1879) (Nakamura 1981), 12 months from hatching to adults in *P. litorale* (Tomaschko et al. 1998; Wilhelm et al. 1997). Mercier et al. (2015) even estimated that up to 7 years could be necessary for the deep-sea species *N. hirtipes* to reach adult size.

The variations in the population composition can also explain the observations made by Le Roux et al. (2020), who counted very few juveniles compared to the number of adults. They would not be mutually exclusive as proposed, but rather result from the periodic phenology of *A. hilgendorfi*. It would be of interest to investigate how distinct and putatively unconnected populations of the same species are synchronized with each other along the year.

Biometry

The measurements of *A. hilgendorfi* in Belgium match those made elsewhere for this species. In Barreto and Avise (2008), the male trunk width was on average 1.49 mm, against 1.5 and 1.6 ± 0.2 mm in the present study; the female trunk width was 1.39 mm against 1.4 and 1.4 mm ± 0.2 here. In both populations, males were wider than females. While the landmark used to measure trunk length was different to this study, they found out that females were longer than males, which was also the case here. A few Japanese *A hilgendorfi* adults were measured at an average length of slightly more than 2.0 mm and an average width of almost 1.5 mm (Nakamura and Fujita 2004) which coincides with the lengths measured here. They also carried out measurements on juveniles of different stages, which roughly ranged from 1.0 to 1.5 mm in length, and 0.5 to 1.0 mm in width. The present study did not discriminate between the developmental stages, yet the overall lengths and widths remained within these ranges. Therefore, *A. hilgendorfi* in Belgium does not seem to have undergone significant changes in size posterior to introduction where such differences may be observed between isolated sub-populations (e.g. Cassel-Lundhagen et al. 2011); or may not (e.g. Fabriciusová et al. 2008).

Interestingly, the average size of each subgroup did not remain constant throughout the year. The end of juvenile peaks was indeed immediately followed by smaller adults (especially noticeable in male lengths and female widths) (Figure 5A, E). Same went with juveniles, which were much smaller when the juvenile peaks started. These observations may seem trivial as younger juveniles would obviously be smaller (Nakamura and Fujita 2004) and adult pycnogonids were already observed growing by peeling off cuticle fragments (Lotz and Bückmann 1968). However, they may as well indicate that adults *A. hilgendorfi* have a short life expectancy, likely not over one year. If adults lived longer, one would indeed expect that the older, bigger ones would tone down, even make disappear, the size fluctuations along the year. Female average length, significantly smaller the second year, might arise from environmental factors, as in the non-native insect Cerambycidae *Psacothea hilaris hilaris* (Pascoe, 1857) (Lupi et al. 2015).

Only a few studies have been carried out on pycnogonid limb autotomy and regeneration (Schimkewitsch and Dogiel 1913; Maruzzo et al. 2005; Fornshell 2019; Brenneis et al. 2023; Petrova and Bogomolova 2023). They observed autotomized legs mostly at the junction between the first two coxae. Next, a blastema forms and molts back as a leg, initially smaller than the others (Petrova and Bogomolova 2023). Although these smaller regenerating legs were also observed in the present study, they were not counted, neither were eventual blastemas on ovigers. The latter were observed in *Nymphon australe* Hodgson, 1902, as well as on chelifores (Fornshell 2019), but adults or close-to-adults *A. hilgendorfi* have them atrophied. In short, only the walking appendages totally absent or reduced to a blastema were counted. In total, 38.5% of the measured specimens were missing at least one leg. This percentage is very close to the 36.4% of the pool of *N. australe* in Fornshell (2019).

Autotomy is described as a defensive mechanism, triggered when animals encounter a predator, are trapped, suffer from an infected appendage (Fleming et al. 2007), or respond to a physical injury (Emberts et al. 2017) (i.e. any threat that can jeopardize the animal survival). Therefore, the presence of autotomized individuals in the Belgian population suggests that Belgian *A. hilgendorfi* encounter threats too. The nature of these threats, however, is still to determine. One possibility would be generalist predators, such as the green crab *Carcinus maenas* (Linnaeus, 1758), although *P. litorale* was shown to release high numbers of ecdysteroids acting as a repellent towards it (Tomaschko 1994). Another one would be the byssus formed by the mussels *M. edulis*, abundant on the wave breakers, in which a fair number of individuals were found trapped. One could also imagine that *A. hilgendorfi* was introduced to Europe alongside its own pathogens, or that some European pathogens acquired the capacity to infect it (Bojko et al. 2021). While these eventual threats might theoretically limit the invasion of *A. hilgendorfi*, autotomy likely helped its establishment. On the other hand, autotomy comes with a cost (Emberts et al. 2019). In sea spiders, the digestive and sexual tracts extend into the legs (Bamber 2010; Brenneis et al. 2023), therefore the survival and/or the reproductive success of autotomized individuals might be compromised. Finally, the fact that more front legs had been autotomized makes sense as they would be the exploratory legs, more often subjected to potential dangers. Further studies on pycnogonid autotomy are necessary to address all these questions.

Larval development

The main instar collected in both months was protonymphon, probably indicating that only a fraction of them survive to the successive molts. The clear difference in abundance from June to July, on the other hand, could arise from similar phenomena in place regarding adult and juvenile densities. If they are gregarious, non-pelagic larvae likely are, and the number of found specimens would greatly depend on the area sampled.

Based on our observations, the *A. hilgendorfi* larva is clearly an ectoparasite, which directly contradicts the information in the literature. Indeed, Russel and Hedgpeth (1990) described that *A. hilgendorfi* was an endoparasite of the hydroid *Eucopella everta* (Clark, 1876) and followed an ontogenetic development totally different from other *Ammothea* spp. (Cano-Sánchez and López-González 2013). Hence, Brenneis et al. (2017) mentioned in their review that *A. hilgendorfi* is the only Ammotheidae with an endoparasitic larva, even if it lacks the typical endoparasitic features, and attributed such a phenomenon to "an independent evolutionary event." In the present study, every larva collected was an ectoparasite. One could argue that they might not belong to *A. hilgendorfi*. However, several arguments reject that statement. First, 97.4% adult sea spiders were *A. hilgendorfi*, making it very unlikely that the larvae belong to another species. Second, the protonymphons hatching from *A. hilgendorfi* clutches under a controlled environment looked identical to the ones collected on hydroids, the presence of spinning spines and complete larval appendages pointing at an ectoparasitic mode of life (Burris 2011). Third, the observed development follows the same path as other *Ammothea* spp., notably *Ammothea carolinensis* Leach, 1814, in which each walking leg progressively appears in successive molts, first as unarticulated primordia, then a six-segmented articulated leg, and finally a totally formed leg (Cano-Sánchez and López-González 2013). This path is totally unrelated to the one described in Russel and Hedgpeth (1990) (i.e. a type 4 protonymphon larva according to Brenneis et al. 2017). Therefore, it appears that the larva described in Russel and Hedgpeth (1990) was actually another species.

The presence of larvae parasitizing native hosts also represents the first direct evidence that *A. hilgendorfi* is established in Belgium. Their number per egg sac, combined with their relatively quick life cycle and polygynandrous behavior (Barreto and Avise 2008), suggests that *A. hilgendorfi* displays an r-strategy (Pianka 1970; Southwood et al. 1974). Since that can lead to rapid adaptations, *A. hilgendorfi* could have the potential to quickly become successful, or at least maintain itself when introduced in a new area, especially in disturbed ecosystems (Devin and Beisel 2007; Sousa et al. 2008; Longo et al. 2012; Ronzhina 2020). Moreover, *A. hilgendorfi* larvae could be observed alive for more than a week in the multiwell plates (i.e. without infesting hydrozoans), showing their resilience and their capacity to remain for days on their fathers before finding a suitable host. Given the fact that larvae were collected on two distinct hydroid species, they might be generalist parasites, and investigating the range of potential hosts, within and outside Belgium, would be relevant to assess their invasiveness.

Finally, Le Roux et al. (2020) mentioned the disappearance of eggs after a few days of maintenance, which was also observed here. However, the egg clutches were not eaten by grown-up specimens, as suggested, they were instead found lying at the bottom of maintenance mesocosms. Under microscopical investigations of some of them, most eggs had hatched, pointing at the fact that larvae fall from the adult males before looking for a host. Further studies would be relevant to determine to exact time eggs hatch after reproduction, as well as the time during which protonymphon larvae remain attached to the ovigers.

Bioinvasion

Not only is *A. hilgendorfi* established in Belgium, but it also developed intensively to the point of becoming locally dominant and completely outnumbering native sea spiders (Figure 2A). Therefore, this population can also be considered as invasive. As invasive species represent the second-biggest threat to marine ecosystems and biodiversity, second to habitat destruction (Sol et al. 2008; Bax et al. 2003; Giangrande et al. 2020) and may also be harmful for health or the economy (Pyšek and Richardson 2010; Cuthbert et al. 2021), assessing their impact has become more and more crucial. The impact of invasive species was defined as the product of their range, their abundance, and the effect per individual (Parker et al. 1999).

The reported range of *A. hilgendorfi* is already wide (Table 1), but the monitoring area should be extended as it may spread even further, notably along the Belgian coast, the North Sea, or elsewhere in Europe. It might actually already be the case, as the other recordings were mostly sporadic, leaving important gaps in its distribution in Europe, translating a lack of investigation rather than an absence of the species in certain regions. Although no larvae have been reported elsewhere in Europe, the presence of ovigerous males in Venice (Krapp and Sconfietti 1983), the Netherlands (Faasse 2013) and Brittany (Le Roux et al. 2020) signals that *A. hilgendorfi* is likely established in these areas as well, even if this is still to demonstrate. Whether the several European sub-populations result from independent episodes of introduction, or are connected to each other, should be checked through population genetics.

Regarding abundance, *A. hilgendorfi* can reach high densities at least in Belgium, meaning that it has the potential to become dominant in other countries as well. The number of individuals collected in the second year being almost 150% the one of the first year, this bioinvasion has unlikely reached its capacity yet. Since no other invasive pycnogonid population has been reported to this day, it is complex to estimate the potential of this bioinvasion without a point of comparison. This species should then be watched out, as its invasion in Europe might only be in the very early stage.

Assessing the effect per individual of an invader is even more difficult when the knowledge on its ecology is as cursory as it is for *A. hilgendorfi*. Further studies should be carried out to address that, but the effects might be numerous. First, they might outcompete other species of the same niche (native Belgian sea spiders have almost disappeared (Daro 1969, 1970); same conclusion in Venice (Mizzan 2018)). Our results suggest that *A. hilgendorfi* has a shorter life cycle and reproduces with a higher frequency than *P. litorale*, which could explain the absence of *P. litorale* in our samples, although known to be formerly present (Daro 1969, 1970). Ecosystems and trophic webs consist of balanced networks of interactions but introduced alien species create new interactions (David et al. 2017;

Britton et al. 2018). A first direct way a non-native species can affect the local trophic web is through predation as the arrival of a new predator in the ecosystem leads to decreases in the native species population that it preys on (David et al. 2017; Britton et al. 2018). In general, Ammotheids have been observed feeding on a wide range of preys (Dietz et al. 2018), and *A. hilgendorfi* was noticed eating several preys (Le Roux et al. 2020). It could then harm sessile communities, since, like most pycnogonids, it is not a predator *sensu stricto* but rather an ectoparasite, sucking up the tissues of their preys and leaving them with a scar, potentially causing health deterioration and death (Mercier and Hamel 1994). As *A. hilgendorfi* is now very abundant in Belgium, it could lead to a decline of the population of these communities. Identifying with precision the diet of this species has thus become crucial to understand its impact.

Eno et al. (1997) state that *A. hilgendorfi* does not have any effect on the ecosystem. However, a case of pycnogonids negatively impacting other organisms has recently been described: Krol et al. (2023) studied the infestation of several coral species by *Callipallene* spp. in captive systems. The sea spiders caused the coral health to decline and eventually die, since the corals were both preyed on by adults and parasitized by larvae. The authors added that coral would unlikely be a first-choice prey in the pycnogonid natural habitat; they probably started to feed on the coral massively because of the absence of other preys and predators in the system. This context shares similarities with bioinvasions like *A. hilgendorfi*. A species newly introduced in an ecosystem might indeed find itself having no predators and eating other preys than in its native habitat (*e.g.* the lionfish *Pterois volitans* (Linnaeus, 1758) or *Pterois miles* (Bennett, 1828) (Río et al. 2023)). The observations of Krol et al. (2023) and the success of *A. hilgendorfi* here observed could point at great adaptation abilities in pycnogonids, and potential impacts that deserve to be investigated.

The vector through which *A. hilgendorfi* arrived in Europe is also uncertain. Some authors attribute its introduction to international shipping (Krapp and Sconfietti 1983) but others consider that it could have traveled alongside the Japanese oyster *M. gigas* (i.e. co-invasion) (Bamber 1985). This bivalve was introduced in Europe in the late 1960s and in Belgium in 1969 where it quickly became established and invasive (Troost 2010). Thus, the invasion of *M. gigas* may have facilitated the invasion of *A. hilgendorfi* as the former is known to have caused numerous over-invasions (Krueger-Hadfield et al. 2017; Gutow and Buschbaum 2019; McAfee and Connell 2021). Le Roux et al. (2020) reported the presence of *M. gigas* in the sampling sites, although they do not consider the oysters to be a likely vector, because no pycnogonids were reported in French oyster farms. We personally contacted some of them. Most indeed never saw *A. hilgendorfi* on their oysters, but a few did: the ones using a more traditional method, in which the oysters are grown directly on the substrate. This makes sense, because *A. hilgendorfi* does not have a pelagic phase in its life cycle, as it parasitizes benthic hydroids, and the adults can only crawl on the substrate. Considering the predominant presence of *A. hilgendorfi* on Belgian oyster reefs, this potential vector should not be neglected, although several European populations may have arrived via different vectors. Further research involving population genetics could help address this hypothesis.

Sea spiders are known to host a variety of epibionts on their exoskeleton (Wambreuse et al. 2021). The bioinvasion of *A. hilgendorfi* might thus lead to a co-invasion of its epibiontic communities. A first pycnognid virus has recently been described (Bojko et al. 2024), and *A. hilgendorfi* might have brought parasites and viruses, which are known to potentially play important roles in bioinvasion processes and impacts, as it may transmit its pathogens to its preys or to other native sea spider species (Chalkowski et al. 2018; Bojko et al. 2021). The consequences of co-introduced pathogens should not be neglected, and several tools already exist to study them and assess their impact (Bojko et al. 2023a, b), which would be of interest to apply on *A. hilgendorfi* in further studies.

Perspectives

The study of invasive species, their dynamic and impact is only increasing but so is the number of alien species introduced globally that represent just as many potential invaders. In fact, biological invaders represent a major threat to ecosystems and biodiversity, already weakened by global change or human activities, and a lot is still to be understood to efficiently predict and maintain biological invasions. The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) identified the invasive species problematic as one of the major drivers of the biodiversity loss crisis and has launched its "Invasive Alien Species Report" on September 4, 2023 which consists of an assessment report on invasive alien species and their control (IPBES 2023; Roy et al. 2024). It is expected that this report will bring new understanding to the invasive species implications and ways of mitigating their impacts, notably bringing new evidence to decision-makers. Although some regulations already exist to control biological invasions, they are still limited and need profound improvements, especially in marine environments, as pointed out by Kleitou et al. (2021). In response to the growing issue represented by IAS, upcoming collaborative initiatives such as GuardIAS (Katsanekavis et al. 2024) aim at gathering more data and developing innovative tools to support more effective prevention, management and decision-making. *Ammothea hilgendorfi* is now a new species to add to the long list of invasive species in Europe and further research needs to be conducted on the ecology and spreading of *A. hilgendorfi* to assess its possible impacts. *Inter alia*, extending the study to other areas will also be crucial to monitor the spreading of the species, notably along the Belgian coast and the North Coast. Whether the several sub-populations in Europe result from independent episodes of introduction or not, as well as determining the vector for introduction, should be checked through population genetics. Finally, the general ecology of the species is still poorly understood, although it might be the most successful sea spider species and also needs to be further investigated.

Authors' contribution

AF and GC conceptualized the research. AF, LS and NG collected the specimens. AF, LS and BD analyzed the specimens and wrote the paper. IE and GC supervised the work.

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Data availability

Species georeferenced records are available at the European Alien Species Information Network: https://easin.jrc.ec.europa.eu/easin/RJD/Download/e5f8c9d4-f819-445bb958-03dccee2fa7d.

References

Arango CP, Wheeler WC (2007) Phylogeny of the sea spiders (Arthropoda, Pycnogonida) based on direct optimization of six loci and morphology. *Cladistics* 23: 255–293, https://doi.org/10.1111/j.1096-0031.2007.00143.x

Arnaud F, Bamber RN (1988) The Biology of Pycnogonida. In: Blaxter JHS, Southward AJ (eds), Advances in Marine Biology, vol 24. Academic Press, pp 1–96, https://doi.org/10.1016/S0065-2881(08)60073-5

Bain B (1991) Some observations on biology and feeding behavior in two southern California pycnogonids. *Bijdragen tot de Dierkunde* 61: 63–64, https://doi.org/10.1163/26660644-06101006

Bain BA, Govedich FR (2004) Courtship and mating behavior in the Pycnogonida (Chelicerata: Class Pycnogonida): a summary. *Invertebrate Reproduction & Development* 46: 63–79, https://doi.org/10.1080/07924259.2004.9652607

Bamber RN (1985) The itinerant sea-spider *Ammothea hilgendorfi* (Böhm) in British waters. *Proceedings of the Hampshire Field Club and Archeological Society* 41: 269–270

Bamber RN (1988) Confirmation of a new record. *Newsletter of the Porcupine marine natural History Society* 4(37): 1–5

Bamber RN (2010) Sea-spiders (Pycnogonida) of the north-east Atlantic: Keys and notes for the identification of the species (2nd ed). Linnean Society of London, 250 pp

Bamber RN (2012) Anthropogenic spread of the immigrant sea-spider *Ammothea hilgendorfi* (Arthropoda: Pycnogonida: Ammotheidae) in UK waters. *Marine Biodiversity Records* 5: e78, https://doi.org/10.1017/feS1755267212000668

Barreto FS, Avise JC (2008) Polygynandry and sexual size dimorphism in the sea spider *Ammothea hilgendorfi* (Pycnogonida: Ammotheidae), a marine arthropod with brood-carrying males. *Molecular Ecology* 17: 4164–4175, https://doi.org/10.1111/j.1365-294X.2008.03895.x

Barreto FS, Avise JC (2010) Quantitative measures of sexual selection reveal no evidence for sex-role reversal in a sea spider with prolonged paternal care. *Proceedings of the Royal Society B: Biological Sciences* 277: 2951–2956, https://doi.org/10.1098/rspb.2010.0311

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W (2003) Marine invasive alien species: A threat to global biodiversity. *Marine Policy* 27: 313–323, https://doi.org/10.1016/S0308-597X(03)00041-1

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* 26: 333–339, https://doi.org/10.1016/j.tree.2011.03.023

Blankley WO, Grindley JR (1985) The intertidal and shallow subtidal food web at Marion Island. In: Siegfried WR, Condy PR, Laws RM (eds), Antarctic nutrient cycles and food webs. Springer, pp 630–636, https://doi.org/10.1007/978-3-642-82275-9_86

Bojko J, Burgess AL, Baker AG, Orr CH (2021) Invasive non-native crustacean symbionts: diversity and impact. *Journal of Invertebrate Pathology* 186: 107482, https://doi.org/10.1016/j.jip.2020.107482

Bojko J, Dunn AM, Blakeslee AM (eds) (2023a) Parasites and biological invasions. CABI, 183 pp https://doi.org/10.1079/9781789248135.0000

Bojko J, Collings AJ, Burgess AL, Goode JA (2023b) Diagnosing invasive parasites. In: Parasites and biological invasions. CABI, GB, pp 8–23, https://doi.org/10.1079/9781789248135.0002

Bojko J, Maxwell JM, Burgess AL, Nicado L, Federici B, Griffiths HJ, Allcock L (2024) An iridovirus from the Antarctic seaspider *Pentanymphon antarcticum* (Pycnogonida). *Antarctic Science* 36: 47–50, https://doi.org/10.1017/S0954102024000063

Bray DF, Bagu J, Koegler P (1993) Comparison of hexamethyldisilazane (HMDS), Peldri II, and critical-point drying methods for scanning electron microscopy of biological specimens. *Microscopy Research and Technique* 26: 489–495, https://doi.org/10.1002/jemt.1070260603

Brenneis G, Bogomolova EV, Arango CP, Krapp F (2017) From egg to "no-body": an overview and revision of developmental pathways in the ancient arthropod lineage Pycnogonida. *Frontiers in Zoology* 14: 6, https://doi.org/10.1186/s12983-017-0192-2

Brenneis G, Frankowski K, Maaß L, Scholtz G (2023) The sea spider *Pycnogonum litorale* overturns the paradigm of the absence of axial regeneration in molting animals. *Proceedings of the National Academy of Sciences* 120: e2217272120, https://doi.org/10.1073/pnas.2217272120

Britton JR, Ruiz-Navarro A, Verreycken H, Amat-Trigo F (2018) Trophic consequences of introduced species: Comparative impacts of increased interspecific versus intraspecific competitive interactions. *Functional Ecology* 32: 486–495, https://doi.org/10.1111/1365-2435.12978

Burris ZP (2011) Larval morphologies and potential developmental modes of eight sea spider species (Arthropoda: Pycnogonida) from the southern Oregon Coast. *Journal of the Marine Biological Association of the United Kingdom* 91: 845–855, https://doi.org/10.1017/S0025315410001554

Cano-Sánchez E, López-González PJ (2013). New data concerning postembryonic development in Antarctic *Ammothea* species (Pycnogonida: Ammotheidae). *Polar Biology* 36: 1175–1193, https://doi.org/10.1007/s00300-013-1338-0

Cano-Sánchez E, López-González PJ (2018) *Ammothea* species (Pycnogonida: Ammotheidae) collected during the Polarstern cruise ANT-XXIX/3 to Antarctic waters, with a description of a new species. *Marine Biology Research* 14: 769–777, https://doi.org/10.1080/17451000.2018.1521524

Carlton JT, Keith I, Ruiz GM (2019) Assessing marine bioinvasions in the Galápagos Islands: implications for conservation biology and marine protected areas. *Aquatic Invasions* 14: 1–20, https://doi.org/10.3391/ai.2019.14.1.01

Cassel-Lundhagen A, Kaňuch P, Low M, Berggren Å (2011) Limited gene flow may enhance adaptation to local optima in isolated populations of the Roesel's bush cricket (*Metrioptera roeselii*). *Journal of Evolutionary Biology* 24: 381–390, https://doi.org/10.1111/j.1420-9101.2010.02174.x

Chalkowski K, Lepczyk CA, Zohdy S (2018) Parasite ecology of invasive species: conceptual framework and new hypotheses. *Trends in Parasitology* 34: 655–663, https://doi.org/10.1016/j.pt.2018.05.008

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define "invasive" species. *Diversity and Distributions* 10: 135–141, https://doi.org/10.1111/j.1366-9516.2004.00061.x

Cuthbert RN, Pattison Z, Taylor NG, Verbrugge L, Diagne C, Ahmed DA, Leroy B, Angulo E, Briski E, Capinha C, Catford JA, Dalu T, Essl F, Gozlan RE, Haubrock PJ, Kourantidou M, Kramer AM, Renault D, Wasserman RJ, Courchamp F (2021) Global economic costs of aquatic invasive alien species. *Science of The Total Environment* 775: 145238, https://doi.org/10.1016/j.scitotenv.2021.145238

Daro MH (1969) Etude écologique d'un brise-lames de la côte belge: 1. Description et zonation des organismes. *In Annales de la Société royale zoologique de Belgique* 99: 111–152

Daro MH (1970) Etude écologique d'un brise-lames de la côte belge: 2. Biologie et développement saisonnier des espèces. *Annales de la Société Royale Zoologique de Belgique = Annalen van de Koninklijke Belgische Vereniging voor Dierkunde* 3: 159–189

David P, Thébault E, Anneville O, Duyck P-F, Chapuis E, Loeuille N (2017) Impacts of invasive species on food webs: a review of empirical data. In: Bohan DA, Dumbrell AJ, Massol F (eds), Advances in Ecological Research, Vol. 56. Academic Press, pp 1–60, https://doi.org/10.1016/bs.aecr.2016.10.001

Devin S, Beisel J-N (2007) Biological and ecological characteristics of invasive species: A gammarid study. *Biological Invasions* 9: 13–24, https://doi.org/10.1007/s10530-006-9001-0

Dietz L, Dömel JS, Leese F, Lehmann T, Melzer RR (2018) Feeding ecology in sea spiders (Arthropoda: Pycnogonida): what do we know? *Frontiers in Zoology* 15: 1–16, https://doi.org/10.1186/s12983-018-0250-4

Emberts Z, Miller CW, Kiehl D, St. Mary CM (2017) Cut your losses: self-amputation of injured limbs increases survival. *Behavioral Ecology* 28: 1047–1054, https://doi.org/10.1093/beheco/arx063

Emberts Z, Escalante I, Bateman PW (2019) The ecology and evolution of autotomy. *Biological Reviews* 94: 1881–1896, https://doi.org/10.1111/brv.12539

Eno NC, Clark RA, Sanderson WG (1997) Non-native marine species in British waters: a review and directory. Joint Nature Conservation Committee, Peterborough, United Kingdom, 152 pp

Faasse M (2013) Further dispersal of the sea-spider *Ammothea hilgendorfi* (Böhm, 1879) in the North Sea to The Netherlands. *BioInvasions Records* 2: 287–289, https://doi.org/10.3391/bir.2013.2.4.04

Faasse M (2020) *Ammothea hilgendorfi*—Zebrazeespin. In: Verleye T, De Raedemaecker F, Vandepitte L, Fockedey N, Lescrauwaet AK, De Pooter D, Mees J (eds), VLIZ Alien Species Consortium, Nietinheemse soorten van het Belgisch deel van de Noordzee en aanpalende estuaria anno 2020. Vlaams instituut voor de zee (VLIZ), pp 139–143

Fabriciusová V, Kaňuch P, Krištín A (2008) Body size patterns of *Pholidoptera frivaldskyi* (Orthoptera) in very isolated populations. *Journal of Orthoptera Research* 17: 171–176, https://doi.org/10.1665/1082-6467-17.2.171

Fage L (1932) Pêches planctoniques à la lumiere effectuees à Banyulssur-Mer et à Concarneau 11. Pycnogonides. *Archives de Zoologie Expérimentale et Générale* 76(14): 249–261

Flandroit A, Faasse M (2024) *Ammothea hilgendorfi*. In: Verleye TJ, De Raedemaecker F, Vandepitte L, Fockedey N, Lescrauwaet A-K, Mees J (eds), Geïntroduceerde niet-inheemse soorten in het Belgisch deel van de Noordzee en aanpalende estuaria anno 2024. Vlaams instituut voor de zee. VLIZ, Oostende, Belgium, pp 155–160

Flandroit A, Simon L, Caulier G (2024) Description of limb anomalies resulting from molt irregularities in *Ammothea hilgendorfi* (Pycnogonida: Ammotheidae). *Arthropoda* 2: 156–168, https://doi.org/10.3390/arthropoda2020012

Fleming PA, Muller D, Bateman PW (2007) Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews* 82: 481–510, https://doi.org/10.1111/j.1469-185X.2007.00020.x

Fornshell JA (2019) Walking leg regeneration observed in three families and four species of antarctic sea spiders. *Arthropods* 8(4): 110

Fornshell JA (2023) Research on sea spiders (Chelicerata; Pycnogonida) in the era of single-ship oceanographic voyages (1870–1915). *Arthropods* 12(4): 260

Giangrande A, Pierri C, Del Pasqua M, Gravili C, Gambi MC, Gravina MF (2020) The Mediterranean in check: Biological invasions in a changing sea. *Marine Ecology* 41: e12583, https://doi.org/10.1111/maec.12583

Giribet G, Edgecombe GD (2019) The phylogeny and evolutionary history of Arthropods. *Current Biology* 29: R592–R602, https://doi.org/10.1016/j.cub.2019.04.057

Grassle JF, Sanders HL, Hessler RR, Rowe GT, McLellan T (1975) Pattern and zonation: A study of the bathyal megafauna using the research submersible Alvin. *Deep Sea Research and Oceanographic Abstracts* 22: 457–481, https://doi.org/10.1016/0011-7471(75)90020-0

Gutow L, Buschbaum C (2019) Invasion of the Wadden Sea by the Pacific oyster (*Magallana gigas*): a risk to ecosystem services? In: Schröter M, Bonn A, Klotz S, Seppelt R, Baessler C (eds), Atlas of ecosystem services: drivers, risks, and societal responses. Springer International Publishing, pp 233–235, https://doi.org/10.1007/978-3-319-96229-0_36

Hayward PJ, Ryland JS (1995) Handbook of the marine fauna of north-west Europe. Oxford University Press, 812 pp

Helfer H (1936) The fishery grounds near Alexandria (Egypt), VII. Pantopoda. *Notes and memoirs, Ministry of Commerce and Industry, Fisheries Research Directorate, Egypt* 16: 1–6

IPBES (2023) Summary for policymakers of the thematic assessment report on invasive alien species and their control of the intergovernmental science-policy platform on biodiversity and ecosystem services. IPBES secretariat, Bonn, Germany, 56 pp, https://doi.org/10.5281/zenodo.7430692

Katsanevakis S, Zaiko A, Olenin S, Costello MJ, Gallardo B, Tricarico E, Adriaens T, Jeschke JM, Sini M, Burke N, Ellinas K, Rutten S, Poursanidis D, Marchini A, Brys R, Raeymaekers JAM, Noé N, Hermoso V, Blaalid R, Lucy FE, Verbrugge LNH, Staehr PAU, Vandepitte L, de Groot D, Elliott M, Reuver M, Maclaren J, Li M, Oldoni D, Mazaris A, Trygonis V, Hablützel PI, Everts T, Pistevos JCA, Dekeyzer S, Kimmig SE, Rickowski FS, Panov VE (2024) GuardIAS – Guarding European Waters from Invasive Alien Species. *Management of Biological Invasions* 15: 701–730, https://doi.org/10.3391/mbi.2024.15.4.14

Kleitou P, Hall-Spencer JM, Savva I, Kletou D, Hadjistylli M, Azzurro E, Katsanevakis S, Antoniou C, Hadjioannou L, Chartosia N, Christou M, Christodoulides Y, Giovos I, Jimenez C, Smeraldo S, Rees SE (2021) The case of Lionfish (*Pterois miles*) in the Mediterranean Sea demonstrates limitations in EU legislation to address marine biological invasions. *Journal of Marine Science and Engineering* 9: 325, https://doi.org/10.3390/jmse9030325

Krapp F, Sconfietti R (1983) *Ammothea hilgendorfi* (Böhm, 1879), an adventitious pycnogonid new for the Mediterranean Sea. *Marine Ecology* 4: 123–132, https://doi.org/10.1111/j.1439-0485.1983.tb00291.x

Krol L, Dunker FH, LaDouceur E, Biswell E, Dilly GF, Delbeek JC, Albright R, López-Nandam EH, Reinbold N, Igel A, Larkin L, Hill J (2023) Milbemycin oxime (interceptor) treatment of pycnogonid sea spider infestation in three species of corals. *Journal of Zoo and Wildlife Medicine* 54: 292–300, https://doi.org/10.1638/2022-0028

Krueger-Hadfield SA, Kollars NM, Strand AE, Byers JE, Shainker SJ, Terada R, Greig TW, Hammann M, Murray DC, Weinberger F, Sotka EE (2017) Genetic identification of source and likely vector of a widespread marine invader. *Ecology and Evolution* 7: 4432–4447, https://doi.org/10.1002/ece3.3001

Laubier L, Sibuet M, Dahl E, Stromberg J-O (1976) Some quantitative results on benthic communities of the deep Norwegian Sea. *Astarte* 9: 61–79

Le Roux A, Gélinaud G, Le Bail Y, Monnat JY, Morel JY, Paraire O, Ros J (2020) Occurrence of *Ammothea hilgendorfi* (Böhm, 1879) a pycnogonid from the north Pacific, in the Étel river. *An aod, les cahiers naturalistes de l'Observatoire marin* 8: 21–32

Le Roux A, Corbrion P, Gully F, Cochu M, Picard C (2022) Nouveaux signalements d'*Ammothea hilgendorfi* (Pycnogonida, Ammotheidae) sur les côtes de Bretagne. *An aod, les cahiers naturalistes de l'Observatoire marin* (*in press*)

Lehmann T, Heß M, Melzer RR (2018) Sense organs in Pycnogonida: a review. *Acta Zoologica* 99: 211–230, https://doi.org/10.1111/azo.12207

Lipkin Y, Safriel U (1971) Intertidal zonation on rocky shores at Mikhmoret (Mediterranean, Israel). *Journal of Ecology* 59: 1–30, https://doi.org/10.2307/2258448

Longo C, Pontassuglia C, Corriero G, Gaino E (2012) Life-cycle traits of *Paraleucilla magna,* a calcareous sponge invasive in a coastal Mediterranean basin. *PLoS ONE* 7: e42392, https://doi.org/10.1371/journal.pone.0042392

Lotz G, Bückmann D. Die Häutung und die Exuvie von *Pycnogonum litorale* (Strøm) (Pantopoda) (1968). *Zoologische Jahrbücher Abteilung für Anatomie Ontogonie der Tiere* 85: 529–536

Lovely EC (2005) The Life History of *Phoxichilidium tubulariae* (Pycnogonida: Phoxichilidiidae). *Northeastern Naturalist* 12: 77–92, https://doi.org/10.1656/1092-6194(2005)012[0077:TLHOPT]2.0.CO;2

Lupi D, Jucker C, Rocco A, Harrison R, Colombo M (2015) Notes on biometric variability in invasive species: the case of *Psacothea hilaris hilaris*. *Bulletin of Insectology* 68: 135–145, https://doi.org/10.3897/zookeys.482.8946

Maruzzo D, Bonato L, Brena C, Fusco G, Minelli A (2005) Appendage loss and regeneration in arthropods: a comparative view. In: Koenemann S, Jenner R (eds), Crustacea and arthropod relationships. CRC Press, pp 215–245, https://doi.org/10.1201/9781420037548

McAfee D, Connell SD (2021) The global fall and rise of oyster reefs. *Frontiers in Ecology and the Environment* 19: 118–125, https://doi.org/10.1002/fee.2291

Mercier A, Hamel JF (1994) Deleterious effects of a pycnogonid on the sea anemone *Bartholomea annulata*. *Canadian Journal of Zoology* 72: 1362–1364, https://doi.org/10.1139/z94-179

Mercier A, Baillon S, Hamel J-F (2015) Life history and feeding biology of the deep-sea pycnogonid *Nymphon hirtipes*. *Deep Sea Research Part I: Oceanographic Research Papers* 106: 1–8, https://doi.org/10.1016/j.dsr.2015.08.015

Miyazaki K (2022) A taxonomic list of sea spiders (Arthropoda, Pycnogonida) from Japanese waters. *Zoological Science* 39: 16–40, https://doi.org/10.2108/zs210077

Mizzan L (2018) New data on the presence and diffusion of the Exotic sea-spider *Ammothea hilgendorfi* (Böhm, 1879) in the Lagoon of Venice (Italy). *Bollettino del Museo di Storia Naturale di Venezia* 69: 43–46

Morgan E (1978) The energy cost of off-shore migration in *Nymphon gracile* (Pycnogonida). *Zoological Journal of the Linnean Society* 63: 171–180, https://doi.org/10.1111/j.1096-3642.1978.tb02096.x

Nakamura K (1981) Post embryonic development of a pycnogonid, *Propallene longiceps*. *Journal of Natural History* 15: 49–62, https://doi.org/10.1080/00222938100770041

Nakamura K, Fujita T (2004) *Ammothea hilgendorfi* (Pycnogonida: Ammotheidae) associated with a sea-star, *Coscinasterias acutispina* (Echinodermata: Asteroidea), from Sagami Bay, Japan. *Species Diversity* 9: 251–258, https://doi.org/10.12782/specdiv.9.251

Ohshima H (1927) Notes on some pycnogons living semiparasitic on holothurians. *Proceedings of the Imperial Academy* 3: 610–613, https://doi.org/10.2183/pjab1912.3.610

Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, Kareiva PM, Williamson MH, Von Holle B, Moyle PB, Byers JE, Goldwasser L (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 319, https://doi.org/10.1023/A:1010034312781

Perez-Ruzafa A, Munilla T (1992) Pycnogonid ecology in the Mar Menor Murcia SW Mediterranean. *Scientia Marina* 56: 21–28

Petrova M, Bogomolova E (2023) Walking leg regeneration in the sea spider *Nymphon brevirostre* Hodge, 1863 (Pycnogonida). *Arthropod Structure & Development* 77: 101310, https://doi.org/10.1016/j.asd.2023.101310

Pianka ER (1970) On r- and K-Selection. *The American Naturalist* 104: 592–597, https://doi.org/10.1086/282697

Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25–55, https://doi.org/10.1146/annurev-environ-033009-095548

Ragkousis M, Abdelali N, Azzurro E, Badreddine A, Bariche M, Bitar G, Crocetta F, Denitto F, Digenis M, El Zrelli R, Ergenler A, Fortič A, Gerovasileiou V, Grimes S, Katsanevakis S, Koçak C, Licchelli C, Loudaros E, Mastrototaro F, Mavrič B, Mavruk S, Miliou A, Montesanto F, Ovalis P, Pontes M, Rabaoui L, Sevingel N, Spinelli A, Tiralongo F, Tsatiris A, Turan C, Vitale D, Yalgin F, Yapici S, Zenetos A (2020) New alien Mediterranean biodiversity records (October 2020). *Mediterranean Marine Science* 21: 631–652

Río L del, Navarro-Martínez ZM, Cobián-Rojas D, Chevalier-Monteagudo PP, Angulo-Valdes JA, Rodriguez-Viera L (2023) Biology and ecology of the lionfish *Pterois volitans*/*Pterois miles* as invasive alien species: A review. *PeerJ* 11: e15728, https://doi.org/10.7717/peerj.15728

Ronzhina DA (2020) Ecological differentiation between invasive and native species of the genus *Epilobium* in riparian ecosystems is associated with plant functional traits. *Russian Journal of Biological Invasions* 11: 132–142, https://doi.org/10.1134/S2075111720020071

Roy HE, Pauchard A, Stoett P, Renard Truong T (2024) IPBES invasive alien species assessment: full report (Version 4). *Zenodo*, https://doi.org/10.5281/ZENODO.7430682

Russel DJ, Hedgpeth JW (1990) Host utilization during ontogeny by two pycnogonid species (*Tanystylum duospinum* and *Ammothea hilgendorfi*) parasitic on the hydroid *Eucopella everta* (Coelenterata: Campanulariidae). *Bijdragen tot de Dierkunde* 60(3/4): 215–224

Sabroux R, Hassanin A, Corbari L (2019) Four times more species of sea spiders (Arthropoda: Pycnogonida) in Martinique Island (Lesser Antilles). *Marine Biodiversity* 49: 1519–1535, https://doi.org/10.1007/s12526-019-00957-9

Schmidt H-W, Bückmann D (1971) Beobachtungen zur Lebensweise von *Pycnogonum litorale* (Ström) (Pantopoda). *Oecologia* 7: 242–248, https://doi.org/10.1007/BF00345214

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675, https://doi:10.1038/nmeth.2089

Schwindt E, Carlton JT, Orensanz JM, Scarabino F, Bortolus A (2020) Past and future of the marine bioinvasions along the Southwestern Atlantic. *Aquatic Invasions* 15: 11–29, https://doi.org/10.3391/ai.2020.15.1.02

Simberloff D, Gibbons L (2004) Now you see them, now you don't! – population crashes of established introduced species. *Biological Invasions* 6: 161–172, https://doi.org/10.1023/B:BINV.0000022133.49752.46

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393–430, https://www.jstor.org/stable/2097054

Sol D, Vilà M, Kühn I (2008) The comparative analysis of historical alien introductions. *Biological Invasions* 10: 1119–1129, https://doi.org/10.1007/s10530-007-9189-7

Sousa R, Nogueira AJA, Gaspar MB, Antunes C, Guilhermino L (2008) Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): Possible implications for ecosystem functioning. *Estuarine, Coastal and Shelf Science* 80: 289–295, https://doi.org/10.1016/j.ecss.2008.08.006

Southwood TRE, May RM, Hassell MP, Conway GR (1974) Ecological strategies and population parameters. *The American Naturalist* 108: 791–804, https://doi.org/10.1086/282955

Stock JH (1958) Pycnogonida from the Mediterranean coast of Israel. *The Bulletin of the Research Council of Israel* 7B(3/4): 137–142

Stoffels RJ, Closs GP, Burns CW (2003) Multiple scales and the relationship between density and spatial aggregation in littoral zone communities. *Oikos* 103: 81–92, https://doi.org/10.1034/j.1600-0706.2003.12453.x

Strayer DL, D'Antonio CM, Essl F, Fowler MS, Geist J, Hilt S, Jarić I, Jöhnk K, Jones CG, Lambin X, Latzka AW, Pergl J, Pyšek P, Robertson P, von Schmalensee M, Stefansson RA, Wright J, Jeschke JM (2017) Boom-bust dynamics in biological invasions: Towards an improved application of the concept. *Ecology Letters* 20: 1337–1350, https://doi.org/10.1111/ele.12822

Schimkewitsch WM, Dogiel V (1913) Ueber Regeneration bei Pantopoden. *Izvestija Rossijskoj akademii nauk. Serija matematičeskaja* 7: 1147–1156

Tomaschko K-H (1994) Ecdysteroids from *Pycnogonum litorale* (Arthropoda, Pantopoda) act as chemical defense against *Carcinus maenas* (Crustacea, Decapoda). *Journal of Chemical Ecology* 20: 1445–1455, https://doi.org/10.1007/BF02059872

Tomaschko K-H, Wilhelm E, Buckmann D (1998) Growth and reproduction of *Pycnogonum litorale* (Pycnogonida) under laboratory conditions. *Oceanographic Literature Review* 3: 510, https://doi.org/10.1007/s002270050201

Troost K (2010) Causes and effects of a highly successful marine invasion: Case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. *Journal of Sea Research* 64: 145–165, https://doi.org/10.1016/j.seares.2010.02.004

Ulman A, Ferrario J, Occhpinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C, Chatzigeorgiou G, Çiçek BA, Deidun A, Ramos-Esplá A, Koçak C, Lorenti M, Martinez-Laiz G, Merlo G, Princisgh E, Scribano G, Marchini A (2017) A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ* 5: e3954, https://doi.org/10.7717/peerj.3954

Utinomi H (1951) On some Pycnogonids from the Sea around Kii Peninsula. *Publications of the Seto Marine Biological Laboratory* 1: 159–168, https://doi.org/10.5134/174448

Wambreuse N, Hamel, J-F, Mercier A (2021) Epibiotic associations with the deep-sea pycnogonid *Nymphon hirtipes* Bell, 1855 as a source of biodiversity. *Deep Sea Research Part I: Oceanographic Research Papers* 178: 103660, https://doi.org/10.1016/j.dsr.2021.103660

Weisser WW, Volkl W, Hassell MP (1997) The importance of adverse weather conditions for behaviour and population ecology of an aphid parasitoid. *Journal of Animal Ecology* 66: 386–400, https://doi.org/10.2307/5984

Wilhelm E, Bückmann D, Tomaschko K-H (1997) Life cycle and population dynamics of *Pycnogonum litorale* (Pycnogonida) in a natural habitat. *Marine Biology* 129: 601–606, https://doi.org/10.1007/s002270050202

Websites and online databases

GBIF Secretariat (2023) *Ammothea hilgendorfi* (Böhm, 1879). GBIF Backbone Taxonomy. Checklist dataset, https://doi.org/10.15468/39omei, https://www.GBIF.org (accessed 22 October 2024)

Bamber RN, El Nagar A, Arango CP (eds) (2024) Pycnobase: World Pycnogonida Database. https://www.marinespecies.org/pycnobase (accessed 29 November 2024)

Costello MJ, Ahyong S, Bieler R, Boudouresque C, Desiderato A, Downey R, Galil BS, Gollasch S, Hutchings P, Kamburska L, Katsanevakis S, Kupriyanova E, Lejeusne C, Ma KCK, Marchini A, Occhipinti A, Pagad S, Panov VE, Poore GCB, Rewicz T, Robinson TB, Rius M, Sobczyk R, Stern N, Turon X, Valls Domedel G, Verleye T, Vieira LM, Willan RC, Yeo Chong Jinn D, Zhan A (2024) World Register of Introduced Marine Species (WRiMS). *Ammothea hilgendorfi* (Böhm, 1879). https://www.marinespecies.org/introduced/aphia.php?p=taxdetails&id=134607 (accessed 29 November 2024)

European Commission - Joint Research Centre - European Alien Species Information Network (EASIN). https://easin.jrc.ec.europa.eu/ (accessed 29 November 2024)

Google Maps (2024) Map of the coast of Knokke. https://www.google.be/maps/@51.3586262,3.2926875,14z (accessed 29 November 2024)

Google Maps (2025) Map of Belgium. https://www.google.be/maps/@51.2039102,3.36675,6z (accessed 02 March 2025)

RStudio Team (2020) RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/

Supplementary material

The following supplementary material is available for this article:

Table S1. Metadata of the collecting events.
Figure S1. Linear regressions of the lengths and width of the collected specimens.
Figure S2. Protonymphons on hydroid hosts.

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