Another brick in the wall: population dynamics of a symbiotic species of *Oxydromus* (Annelida, Hesionidae), described as new based on morphometry

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Abstract

Oxydromus humesi is an annelid polychaete living as a strict bivalve endosymbiont (likely parasitic) of *Tellina nymphalis* in Congolese mangrove swamps and of *Scrobicularia plana* and *Macomopsis pellucida* in Iberian saltmarshes. The Congolese and Iberian polychaete populations were previously considered as belonging to the same species, the latter showing regular distribution, intra-specific aggressive behaviour, and complex host-entering behaviour. The fresh Iberian samples enabled us to undertake consistent morphometric analyses, as well as to further analyse the characteristics of the association and the population dynamics of the Iberian population hosted by *S. plana*. Among the morphological differences between the Congolese and Iberian specimens, leading to the description of the latter as *Oxydromus okupa* sp. nov., the most important are: 1) longer cephalic appendages, 2) greater distance between the eyes, 3) larger dorsal cirrostyle in relation to the corresponding dorsal lobe and cirrophore. Moreover, dorsal and ventral lobes are similar in length, with the tip of the former reaching the tip of the latter in *O. okupa* sp. nov., while the dorsal lobe is much shorter than the ventral one in *O. humesi*. Mature adults of *O. okupa* sp. nov. occurred during the whole study period, with a higher percentage of ripe females in spring and, particularly, in summer. Numerous host specimens showed the symbiont's most preferred shell length (>26 - 36 mm). However, the prevalence was very low (usually <5%) and showed a clear seasonal pattern, being lower during spring/summer. This suggests that males are able to leave their hosts during this period, most likely to improve fertilization by directly entering or approaching a host occupied by a ripe female, while females usually remain inside. Based on the new results, the current knowledge of symbiotic Hesionidae and their relationships with invertebrate hosts is updated and discussed.

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Introduction

The Hesionidae is a well-known family of polychaetes whose representatives are most often free-living in shallow waters, although some occur in the deep-sea (Summers *et al.*, 2015) and some lives as symbionts of other invertebrates (Martin and Britayev, 1998; Miller and Wolf, 2008; De Assis *et al.*, 2012; Martin *et al.*, 2012, 2015; Britayev *et al.*, 2013; Chim *et al.*, 2013).

Oxydromus humesi (Pettibone, 1961), which is one of these symbiotic hesionids, has been reported as a strict bivalve-associate that is hosted by *Tellina nymphalis* Lamarck, 1818 in Loango (Congo mangrove swamps) and by *Scrobicularia plana* (Da Costa, 1778) and *Macomopsis pellucida* (Spengler, 1798) (reported as *Macoma cumana* (O.G. Costa, 1830)) in Cádiz Bay, Iberian Peninsula (Pettibone, 1961; Martin *et al.*, 2012, 2015). The Iberian hosts show similar morphologies and modes of life. However, in Cádiz Bay, *M. pellucida*

Fig. 1. *Oxydromus okupa* sp. nov. Schemes of anterior end (a), two mid-body segments (b) and one mid-body parapodia (c), showing the measurements used in the morphometric analyses. WW: worm width with parapodia (μm); WWP: worm width without parapodia (μm); HW: head width (μm); HL: head length (μm); LA: length of lateral antenna (μm); PP: length of palpophore (μm); PS: length of palpostyle (μm); DAE: distance between anterior eyes (μm); DPE: distance between posterior eyes (μm); DAE: distance between anterior and posterior eyes (μm); DL: length of dorsal lobe (μm); DCP: length of dorsal cirrophore (μm); DCS: length of dorsal cirrostyle (μm); PNCL: length of posterior neurochaetal lobe (μm); VL: ventral lobe; VC: length of ventral cirri (μm); L: long cirrostyle; S: short cirrostyle.

occurs mainly in the subtidal (E. Pascual, pers. observ.), whilst it is very rare in the intertidal (Subida *et al.*, 2013). In turn, *S. plana* occurs in intertidal salt-marshes (like Río San Pedro), where it shows very high abundances (Subida *et al.*, 2011; Drake *et al.*, 2014).

Based on occasional sampling of mainly *S. plana* associates, it has been inferred that the association apparently affects negatively the metabolism of the host (and so it is considered to be closer to a parasitism), that infestations did not occur in hosts measuring less than 20 mm in shell length, that all symbionts seem to be adults (which are not sexually dimorphic), and that there are no relationships between worm's size and shell length (Martin *et al.*, 2012, 2015). However, nothing is known of the seasonal characteristics of the association, as well as of the life cycle of the symbiont. Also, when re-describing *O. humesi*, Martin *et al.* (2015) discussed the possibility of having different species at the two known locations due to an hypothetical biogeographic isolation of the respective populations. The fact that no fresh specimens were available from the Congolese population (i.e. the original specimens were formalin-fixed) prevented a molecular approach. Morphologically, animals of the two known populations ap-

peared to be indistinguishable and a preliminary comparison of chaetal morphology and appendage measurements based on the available specimens was therefore inconclusive (Martin *et al.*, 2015), which lead us to undertake a more robust study based on morphometry.

Therefore, the following questions were addressed: Were hosts < 20 mm infested during the seasonal cycle? Did the symbionts prefer any host size among those > 20 mm? Were the prevalence and intensity of infestation constant along time? Had the symbiont a seasonal trend in reproductive traits or size-class structure? These observations were compared with the few available data on symbionts infesting *M. pellucida*. Based on the new results, the current knowledge on the symbiotic relationships involving hesionid polychaetes worldwide is summarized and discussed.

Material and Methods

Collection details and sampling were explained by Martin *et al.* (2015). Specimens of the host bivalves were collected: monthly, from April 2011 to May 2012 and in January 2013, at Río San Pedro, 36°31'56.28",

6°12'53.28" (*S. plana*); in January 2013 at Río San Pedro mouth, 36°31'47.28", 6°14'52.80" (*M. pellucida*). The specimens of *S. plana* from May 2011 were damaged during sampling. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Possible non-biotic influences in the observed patterns could not be determined because no environmental data were recorded during the study period.

In the laboratory, the collected specimens of *S. plana* and *M. pellucida* were opened to estimate the infestation intensity and prevalence. All obtained worms, and the bivalves harbouring them, were counted. The longest shell diameter of the hosts was measured (length, in mm) using callipers. Samples of *S. plana* (i.e. 100 to 300 specimens) were collected monthly and measured (independently of the presence of the symbiont) to define the seasonal size structure of the host population.

The width (in μm) of the tenth segment (parapodia included) was used as a proxy for symbiont size (WW). WW was measured under a Nikon SMZ645 stereomicroscope equipped with a micrometric ocular. Ripe females were identified by the presence of intracoelomic oocytes, which were usually visible through the body wall. In preserved specimens, the worm tissues become more opaque than in living ones, which usually hindered observation of the oocytes. Their presence was confirmed by placing the worms in a Petri dish and then cutting their body wall and applying a gentle pressure with the forceps so that present oocytes would spill from the coelomic cavity for observation. Digital images were taken with a CMEX camera, linked to a Zeiss Stemi 2000-c, stereomicroscope, using the ImageFocus 4.0 software by Euromex.

The sizes of the Iberian symbionts and their hosts were used to estimate their monthly size-class frequencies. The frequency of infested bivalves and that of the whole population was estimated separately and then expressed as percentages for comparison. The worm/host size and the ripe females/prevalence relationships were assessed by correlation analyses, while differences in percentage of females and prevalence during warm (i.e. March to September) and cold (i.e. October to February) periods were assessed by one-way Analysis of Variance (one-way ANOVA).

For morphometric purposes, preserved specimens of the Congolese population were kindly loaned by the United States National Museum of the Smithsonian Institution (USNM). Twenty-five specimens from the Iberian population, representative of all available sizes, were selected for the analyses, while for the Congolese

population, 25 specimens were selected on the basis of the quality of the available specimens (as all of them were similar in size). The following characters were selected and measured (Fig. 1): WW, worm width without parapodia (WWP, μm), worm length (WL, μm), number of segments (NS), head width (HW, μm), head length (HL, μm), length of lateral antenna (LA, μm), length of palpophore (PP, μm), length of palpostyle (PS, μm), distance between anterior eyes (DAE, μm), distance between posterior eyes (DPE, μm), distance between anterior and posterior eyes (DAPE, μm), length of dorsal lobe (DL, μm), length of dorsal cirrophore (DCP, μm), length of dorsal cirrostyle (DCS, μm), length of posterior neurochaetal lobe (PNCL, μm), and length of ventral cirri (VC, μm) (measured at the 10th parapodia level). As cirrostyles alternate short and long along the body (Martin *et al.*, 2015), the measurements were recorded for two parapodia (from chaetiger 10 to 30, depending on the specimen) bearing long and short cirrostyles, respectively, and indicated by adding L and S to the end of the acronym (e.g. "DCSL" and "DCSS" meaning dorsal cirrostyle from parapodia having long and short cirrostyles, respectively). Special care was addressed to avoid measurements on damaged appendages. The eyespot diameter, which is highly variable among the specimens of the two species (probably as a result to different individual responses to fixation) was not considered a valid taxonomic character and was not used in the statistical analyses. This also led us to measure all characters based on the eyes at the centre of each eyespot (Fig. 1). WWP, WL and NS were measured as WW, while the remaining characters were measured under a Motic BA210 binocular microscope equipped with a TOUPCAM™ U3CMOS digital camera, managed through the ToupView 3.7 software.

The inter-population differences were analysed for three different datasets: 1) raw data (direct measurements), 2) size-independent data; 3) taxonomically relevant character proportions (i.e., WL/WW, NS/WW, WWP/WW, DCPL/DLL, DCSL/DLL, DCSL/DCPL, VCL/PNCLL, DCPS/DLS, DCSS/DLS, DCSS/DCPS, VCS/PNCLS, HL/HW, LA/HL, PP/HL, PS/HL, PS/PP, DAE/HW, DPE/HW, DAPE/HL, DAE/DPE). Size-dependency of both measured characters and character proportions was assessed by Pearson correlation. Size-independent measurements were used without transformation, while size-dependent ones were divided by worm size (WW). The inter-population differences for the averaged measurements were estimated by one-way ANOVAs. Since multiple F-tests were carried out within each data set, the level of statistical significance was set

Fig. 2. Principal Component Analyses plots. a. Based on raw data. b. Based on size independent data. c. Based on measurement proportions.

the new species was based on the size-independent data as well as on the character proportions.

All character sets (i.e., raw data, size-independent

Table 1. Relationships with worm size (as body width with parapodia) in *Oxydromus okupa* sp. nov., with bold characters indicating significant differences. A. Morphometric measurements. B. Taxonomically relevant proportions. Measurement abbreviations as in Fig. 1. Coeff: Pearson correlation coefficient; p: significance level.

A. Measurements	Iberian		Congolesse		B. Proportions	Iberian		Congolesse	
	Coeff	p	Coeff	p		Coeff	p	Coeff	p
WL	0.873	< 0.0001	0.784	< 0.0001	WL/WW	0.608	0.001	0.369	0.091
NS	0.749	< 0.0001	0.529	0.011	NS/WW	0.056	0.789	-0.542	0.009
WWP	0.846	< 0.0001	0.600	0.003	WWP/WW	0.219	0.293	-0.200	0.373
DLL	0.863	< 0.0001	0.289	0.193	DCPL/DLL	0.104	0.619	0.158	0.483
DCPL	0.777	< 0.0001	0.341	0.121	DSCL/DLL	-0.476	0.016	0.144	0.52
DCSL	0.835	< 0.0001	0.365	0.094	DSCL/DCPL	-0.489	0.013	-0.036	0.872
PNCLL	0.780	< 0.0001	0.211	0.346	VCL/PNCL	-0.170	0.418	0.183	0.416
VCL	0.625	0.001	0.597	0.003	DCPS/DLS	0.189	0.366	-0.100	0.65
DLS	0.835	< 0.0001	0.504	0.017	DSCS/DLS	-0.478	0.016	0.003	0.989
DCPS	0.716	< 0.0001	0.164	0.466	DSCL/DCPL	-0.438	0.028	0.062	0.783
DCSS	0.796	< 0.0001	0.384	0.078	VCS/PNCS	-0.066	0.755	0.207	0.356
PNCLS	0.713	< 0.0001	0.223	0.319	HL/HW	-0.564	0.003	0.103	0.647
VCS	0.693	0.000	0.574	0.005	LA/HL	-0.379	0.062	0.077	0.732
HW	0.896	< 0.0001	0.239	0.283	PP/HL	0.151	0.472	-0.067	0.767
HL	0.646	0.000	0.319	0.147	PS/HL	-0.472	0.017	-0.363	0.096
LA	0.099	0.637	0.215	0.336	PS/PP	-0.498	0.011	-0.256	0.250
PP	0.654	0.000	0.278	0.210	DAE/HW	-0.212	0.310	-0.364	0.096
PS	0.086	0.684	-0.120	0.595	DPE/HW	-0.106	0.614	-0.274	0.218
DAE	0.860	< 0.0001	0.090	0.691	DAPE/HL	0.729	< 0.0001	0.080	0.722
DPE	0.878	< 0.0001	0.099	0.663	DAE/DPE	-0.100	0.636	0.003	0.990
DAPE	0.809	< 0.0001	0.337	0.126					

data, proportions) were analysed by Principal Component Analysis (PCA) based on normalised data. The significance of the inter-population differences was explored by one-way analysis of similarity (ANOSIM) based on Euclidean distance resemblance matrices, while the contribution of each measured character to the distance within and between the two populations was assessed by the Similarity Percentages analysis (SIMPER) based on Euclidean distance. Forward step-wise discriminant analyses (FSDA) were used to obtain discriminant functions for the two populations under study, using the three datasets and assuming equal intra-population covariance matrices and threshold significance levels of 0.05 both to include and to remove variables. The similarity of the intra-population covariance matrices was assessed by the Box test (based on an asymptotic approach to the Fisher's F index) and the differences between population-averaged vectors were assessed by the Rao approach to the Wilks' Lambda test. The classification matrices were built by considering the sensitivity percentage (i.e. well classified positive events) as belonging to the Iberian

population, and the specificity percentage (i.e., well classified negative events) as belonging to the Congolesse population. Furthermore, to determine the probability that one of the observed specimens would effectively belong to one of the two populations, we also performed a cross-validation in which each observation was removed from the original matrix and the model and the forecast successively estimated, allowing a better adjust to the model (Huberty, 1994).

Pearson correlation analyses, one-way ANOVAs and discriminant analyses were performed with the XLSTAT software (2015.5.01.23039, copyright by Adinsoft 1995-2016). All remaining analyses were conducted using the PRIMER software, version 6.1.11, copyright by PRIMER-E Ltd. 2008 (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

Iberian	Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
PS	0.597	0.672	0.51	2.30	2.30
LA	0.648	0.68	0.41	2.33	4.63
PP	0.587	0.97	0.52	3.32	7.95
DAPE	0.522	1.09	0.49	3.73	11.68
DCSS	0.397	1.1	0.48	3.76	15.44
DCSL	0.324	1.18	0.50	4.05	19.49
DLS	-0.424	1.21	0.47	4.14	23.63
HL	0.267	1.26	0.49	4.31	27.94
VCS	0.383	1.35	0.53	4.62	32.56
DAE	0.376	1.39	0.50	4.76	37.32

Congolese					
NS	0.46	0.13	0.43	1.83	1.83
WL	-0.201	0.168	0.45	2.36	4.19
PP	-0.667	0.206	0.51	2.91	7.10
VCL	-0.386	0.212	0.53	2.99	10.08
WW	-8.21E-2	0.214	0.52	3.01	13.09
DPE	-0.283	0.242	0.47	3.41	16.50
DAE	-0.427	0.242	0.47	3.41	19.91
WWP	0.319	0.242	0.53	3.41	23.32
PNCLL	-0.377	0.245	0.46	3.45	26.77
DAPE	-0.593	0.255	0.51	3.58	30.35

	Iberian Av.Value	Congolese Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
LA	0.648	-0.737	2.9	0.88	5.96	5.96
PS	0.597	-0.678	2.76	0.80	5.68	11.64
PP	0.587	-0.667	2.7	0.95	5.55	17.18
DAPE	0.522	-0.593	2.53	0.89	5.20	22.38
DLS	-0.424	0.482	2.32	0.78	4.76	27.15
DCSS	0.397	-0.451	2.28	0.95	4.69	31.84
NS	-0.405	0.46	2.25	0.73	4.63	36.47
VCS	0.383	-0.435	2.23	0.89	4.58	41.04
DAE	0.376	-0.427	2.21	0.97	4.54	45.59
DCSL	0.324	-0.368	2.16	0.88	4.44	50.03

Table 2. List of the ten most contributing raw measurements to the intra-population similarities and inter-population dissimilarity based on the SIMPER analyses. Measurement abbreviations as in Fig. 1. Av.Value: Average value; Av.Sq.Distance: average square distance; Sq.Distance/SD: square distance divided by standard deviation; Contrib%: percentage of contribution; Cum.%: cumulative percentage of contribution.

Results

Morphometry

Raw data. The PCA based on raw data shows a strong size-dependency of most characters, with the first axis (Eigenvalue = 12.0) explaining 54.6% of the variation and the second one (Eigenvalue = 3.52) only 16.0% (Fig. 2A). All characters of the Iberian population were size-dependent (except LA and PS), while the only size-dependent characters in the Congolese population were WL, NS, WWP, VCL, DLS and VCS (Table 1A). The size-relationship in the PCA plot was also more evident for the Iberian population, whose specimens showed a wider size-range representativeness than the

Congolese ones. Nevertheless, the two populations clearly form separate groups in the PCA plot, and turned to be significantly different (ANOSIM, Global $R = 0.435$, significance level = 0.1%).

The intra-population average distances for the Iberian and Congolese worms were 29.9% and 7.10%, respectively, whilst the inter-population dissimilarity was 48.67%. PS, LA and PP, and NS, WL and PP most contributed to the Iberian and Congolese intra-population similarity, respectively, whilst LA, PS and PP most contributed to the inter-population dissimilarity (SIMPER, Table 2).

All averaged character measurements showing significant differences were higher in the Iberian than in the Congolese population, except for NS and DLS, with

Table 3. Comparative table of the raw morphometric measurements (μm) in the Iberian and Congolese populations. Differences expressed as percentages, with bold characters indicating significant differences (according to Benjamini & Hochberg, 1995) that may be higher (normal text) or lower (italics) in the Iberian than in the Congolese population, respectively. Measurement abbreviations as in Fig. 1. Min: minimum; Max: maximum; Mean: average $\pm$ standard deviation; F: Fisher's F index; p: significance level.

	Iberian Population			Congolese Population			ANOVA		Difference	
	Min	Max	Mean	Min	Max	Mean	F	p	%	
WW	1875.00	3443.00	2633.27 $\pm$ 497.83	2250.00	2866.67	2536.67 $\pm$ 194.86	0.816	0.371	1.9	
WL	8000.00	33435.90	20819.19 $\pm$ 7604.17	15139.24	24820.51	18611.64 $\pm$ 2389.06	1.816	0.184	5.6	
NS	29.00	76.00	56.58 $\pm$ 14.11	60.00	78.00	66.77 $\pm$ 4.24	11.303	0.002	8.3	
WWP	604.17	1525.00	1055.33 $\pm$ 263.12	925.00	1375.00	1170.00 $\pm$ 119.02	3.941	0.053	5.2	
DLL	154.14	492.73	337.78 $\pm$ 100.82	316.78	473.84	378.92 $\pm$ 39.21	3.615	0.063	5.7	
DCPL	29.78	136.99	66.94 $\pm$ 25.25	36.04	84.25	65.63 $\pm$ 13.55	0.052	0.820	1.0	
DCSL	398.45	913.87	663.81 $\pm$ 136.56	418.99	869.06	565.64 $\pm$ 94.49	8.737	0.005	8.0	
PNCLL	113.96	315.28	212.31 $\pm$ 65.01	90.26	229.40	168.23 $\pm$ 31.26	9.334	0.004	11.6	
VCL	155.63	387.73	259.18 $\pm$ 69.38	177.06	258.60	216.29 $\pm$ 27.29	8.271	0.006	9.0	
DLS	125.32	517.34	328.61 $\pm$ 95.41	331.03	502.24	406.43 $\pm$ 49.45	13.108	0.001	10.6	
DCPS	26.45	118.90	62.82 $\pm$ 25.11	37.83	109.78	64.16 $\pm$ 15.52	0.051	0.822	1.1	
DCSS	344.68	801.26	603.00 $\pm$ 122.10	366.63	705.07	493.54 $\pm$ 85.87	13.442	0.001	10.0	
PNCLS	85.78	322.40	208.02 $\pm$ 63.89	90.84	222.50	164.20 $\pm$ 31.51	9.458	0.003	11.8	
VCS	158.33	374.15	262.02 $\pm$ 64.97	165.31	270.01	216.46 $\pm$ 28.19	8.271	0.006	9.5	
HW	411.57	785.31	614.68 $\pm$ 108.05	448.16	654.12	550.64 $\pm$ 49.60	7.253	0.010	5.5	
HL	236.14	402.91	331.47 $\pm$ 47.24	231.31	371.43	306.69 $\pm$ 30.01	4.900	0.032	3.9	
LA	133.55	360.21	235.76 $\pm$ 45.30	105.40	220.68	159.05 $\pm$ 31.87	47.964	< 0.0001	19.4	
PP	71.72	136.80	101.67 $\pm$ 18.56	62.44	93.26	77.28 $\pm$ 8.32	35.945	< 0.0001	13.6	
PS	130.57	223.77	168.91 $\pm$ 27.77	61.88	165.44	126.68 $\pm$ 23.65	33.523	< 0.0001	14.3	
DAE	322.40	621.90	503.43 $\pm$ 85.83	390.00	524.90	442.24 $\pm$ 35.70	10.831	0.002	6.5	
DPE	287.10	604.40	452.76 $\pm$ 79.49	370.30	493.10	416.02 $\pm$ 30.37	4.663	0.036	4.2	
DAPE	46.02	118.40	83.49 $\pm$ 18.41	50.27	79.48	64.29 $\pm$ 8.61	22.321	< 0.0001	13.0	

Raw data	F	Lambda	p	Coeff.	Congo	Iberian
Intercept					-21.601	-41.824
DLS	12.366	0.784	0.001	-1.417	0.022	-0.062
PP	31.334	0.590	< 0.0001	0.916	0.162	0.451
PNCLL	6.914	0.867	0.012	0.716	0.042	0.108
LA	44.014	0.506	< 0.0001	0.548	0.080	0.144
Size independent						
Intercept					-83.702	-92.545
WL	1.365	0.971	0.249	1.023	-3.634	0.633
NS	29.795	0.602	< 0.0001	-0.967	3471.027	1471.413
DLS	36.121	0.555	< 0.0001	-0.832	254.506	7.696
PP	37.264	0.547	< 0.0001	0.754	537.571	1448.983
PNCLL	9.920	0.819	0.003	0.471	417.520	630.082
LA	44.014	0.506	< 0.0001	0.400	0.107	0.167
Proportions						
Intercept					-397.626	-482.539
WL/WW	1.365	0.971	0.249	1.473	9.379	14.975
NS/WW	29.795	0.602	< 0.0001	1.120	141.420	-1968.232
DCSS/DLS	48.629	0.481	< 0.0001	0.880	39.606	53.963
PS/HL	20.926	0.683	< 0.0001	0.639	88.314	130.671
DAE/DPE	16.763	0.729	0.0002	0.371	599.499	646.260

Table 4. Results of the discriminant analyses based on raw data, size-independent data and measurement proportions. F: Fisher's F index; Lambda: Rao approach to the Wilks' Lambda test; p: significance level; Coeff.: Standardized coefficients for the variables included in the inter-population discriminant functions, arranged in a decreasing order, according to their contribution to the inter-population discrimination; Congo, Iberian: coefficients of the selected variables in the classification functions for the Congolese and Iberian populations, respectively. Intercept: Intercept of the classification functions for each dataset. Measurement abbreviations as in Fig. 1.

Fig. 3. Scatterplots for individual scores and centroids of the discriminant functions obtained by the discriminant analyses carried out on the three datasets: raw data, size-independent data and measurement proportions. IB: Iberian population; CO: Congolese population.

the most remarkable difference being at LA (one-way ANOVA, Table 3).

Based on raw data, the discriminant function included the variables DLS, PP, PNCLL and LA (Table 4). This function correctly classified the 100% of individuals from both localities (Fig. 3) and the success probability in the cross validation was of 98%.

Size-independent data. For the purpose of this analysis, and taking into account that the size range was well represented in the Iberian population, only LA and PS raw data were considered as size-independent (Table 1A). The PCA plot based on size-independent data revealed again two clearly different groups corresponding to the two studied populations (Fig. 2B). Despite the axes being less representative than those obtained for the raw data (Eigenvalues = 6.61 and 3.49, variation explained = 31.5% and 16.6%, respectively for axis 1 and 2), the two populations were more clearly distinguishable and showed more significant differences than the raw data (ANOSIM, global $R = 0.539$, significance level = 0.1%).

Based on these size-independent characters, the intra-population average distance within the Iberian and Congolese populations was 21.0% and 11.1%, respectively, whilst the inter-population distance was 50.3%. PS, DAPE and LA, and WL, NS, VCS, and PP most contributed to the Iberian and Congolese intra-population similarity, respectively, whilst the Congolese vs.

Iberian dissimilarity was mainly explained by LA, DAPE, DLS, PP, and PS (SIMPER, Table 5).

When comparing the averaged character measurements, all those showing significant differences were higher in the Iberian than in the Congolese population (particularly, LA, PS, PP and DAPE), except for NS, WWP, DLL, and DLS in particular (one-way ANOVA, Table 6).

Based on size-independent data, the discriminant function included the variables WL, NS, DLS, PP, PNCLL, and LA (Table 4). This function correctly classified the 100% of individuals from both localities (Fig. 3) and the success probability in the cross validation was 100%.

Character proportions. Nine of the 20 character proportions analysed showed significant negative ($n = 7$) or positive ($n = 2$) correlations with size, indicating allometric relationships in the Iberian population, while only one was significantly negatively correlated with size in the case of the Congolese population (Table 1B).

In the PCA based on character proportions, Axes 1 (eigenvalue = 5.38) and 2 (eigenvalue = 3.13) explained 26.9% and 15.6% of the variation, respectively. The PCA plot also highlighted a marked clustering for the individuals of the two populations under study (Fig. 2C), the results being slightly less discriminant than the previous ones but equally highly significant (ANOSIM, Global $R = 0.421$, significance level = 0.1%).

Table 5. List of the ten most contributing size-independent measurements to the intra-population similarities and inter-population dissimilarity based on the SIMPER analyses. Measurement abbreviations as in Fig. 1. Av.Value: Average value; Av.Sq.Distance: average square distance; Sq.Distance/SD: square distance divided by standard deviation; Contrib%: percentage of contribution; Cum.%: cumulative percentage of contribution.

Iberian	Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
PS	0.597	0.672	0.51	3.20	3.20
DAPE	0.63	0.675	0.43	3.21	6.42
LA	0.648	0.68	0.41	3.24	9.66
DLS	-0.616	0.686	0.44	3.27	12.92
DCSL	0.386	0.789	0.48	3.76	16.68
DCSS	0.463	0.801	0.47	3.82	20.50
HW	0.379	0.808	0.49	3.85	24.35
PP	0.618	0.829	0.46	3.95	28.31
DAE	0.434	0.886	0.49	4.22	32.52
WWP	-0.527	0.977	0.49	4.66	37.18

Congolese	Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
WL	-0.176	0.175	0.47	1.58	1.58
NS	0.657	0.209	0.44	1.88	3.46
VCL	-0.442	0.23	0.53	2.07	5.53
PP	-0.703	0.27	0.49	2.43	7.95
VCS	-0.51	0.32	0.50	2.87	10.83
LA	-0.737	0.344	0.51	3.10	13.92
WWP	0.598	0.369	0.51	3.32	17.24
DAPE	-0.716	0.41	0.51	3.68	20.92
DLS	0.7	0.441	0.51	3.97	24.89
PNCLS	-0.438	0.492	0.53	4.43	29.32

Variable	Iberian Av.Value	Congolese Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
LA	0.648	-0.737	2.9	0.88	5.76	5.76
DAPE	0.63	-0.716	2.85	0.89	5.67	11.43
DLS	-0.616	0.7	2.81	0.81	5.59	17.02
PP	0.618	-0.703	2.8	0.87	5.56	22.58
PS	0.597	-0.678	2.76	0.80	5.49	28.08
NS	-0.578	0.657	2.67	0.81	5.32	33.39
WWP	-0.527	0.598	2.56	0.84	5.08	38.47
DCSS	0.463	-0.526	2.44	0.85	4.86	43.33
DAE	0.434	-0.493	2.38	0.80	4.73	48.05
VCS	0.449	-0.51	2.36	0.78	4.70	52.75

The average intra-population distance for the Iberian and Congolese populations were 21.4% and 11.1%, respectively, whilst the average inter-population distance was 46.0%. DAPE/HL, DCSS/DLS, and LA/HL, and WL/WW, NS/WW, DCSS/DLS, and LA/LH were the most informative proportions for the intra-population similarity in Iberian and Congolese worms, respectively, whilst the intra-population dissimilarity was mainly explained by DCSL/DLL, LA/HL, NS/NW, and DAPE/HL (SIMPER, Table 7).

All averaged character proportions showing signifi-

cant differences, except for NS/WW and WWP/WW, were higher in the Iberian than in the Congolese population, most of them with differences higher than 10% and particularly higher than 20% in the case of DCSS/DLS (one-way ANOVA, Table 8).

Based on character proportions, the discriminant function included the variables WL/WW, NS/WW, DCSS/DLS, PS/HL, and DAE/DPE (Table 4). This function correctly classified the 100% of individuals from both localities (Fig. 3) and the success probability in the cross validation was 96%.

Table 6. Comparative table of the size-independent morphometric measurements in the Iberian and Congolese populations. Differences expressed as percentages, with bold characters indicating significant differences (according to Benjamini and Hochberg, 1995) that may be higher (normal text) or lower (italics) in the Iberian than in the Congolese population. Measurement abbreviations as in Fig. 1. Min: minimum; Max: maximum; Mean: average $\pm$ standard deviation ; F: Fisher's F index; p: significance level.

	Iberian Population			Congolese Population			ANOVA		Difference
	Min	Max	Mean	Min	Max	Mean	F	p	%
WL	4.129	10.449	7.696 $\pm$ 1.903	6.154	8.658	7.214 $\pm$ 0.609	1.379	0.246	3.2
NS	0.014	0.027	0.021 $\pm$ 0.004	0.023	0.031	0.026 $\pm$ 0.002	30.385	< 0.0001	9.6
WWP	0.284	0.492	0.399 $\pm$ 0.054	0.403	0.578	0.462 $\pm$ 0.041	21.926	< 0.0001	7.3
DLL	0.080	0.159	0.127 $\pm$ 0.023	0.122	0.189	0.150 $\pm$ 0.017	16.696	0.000	8.5
DCPL	0.015	0.040	0.025 $\pm$ 0.006	0.016	0.035	0.026 $\pm$ 0.005	0.322	0.573	1.8
DCSL	0.184	0.309	0.253 $\pm$ 0.031	0.167	0.307	0.223 $\pm$ 0.031	11.463	0.001	6.2
PNCLL	0.037	0.110	0.080 $\pm$ 0.015	0.040	0.086	0.066 $\pm$ 0.011	12.614	0.001	9.3
VCL	0.059	0.143	0.099 $\pm$ 0.020	0.073	0.112	0.085 $\pm$ 0.009	9.067	0.004	7.2
DLS	0.065	0.162	0.123 $\pm$ 0.022	0.132	0.197	0.161 $\pm$ 0.018	42.182	< 0.0001	13.1
DCPS	0.014	0.036	0.023 $\pm$ 0.007	0.016	0.042	0.025 $\pm$ 0.006	1.095	0.301	3.9
DCSS	0.154	0.279	0.230 $\pm$ 0.031	0.142	0.268	0.194 $\pm$ 0.028	18.012	< 0.0001	8.4
PNCLS	0.032	0.112	0.078 $\pm$ 0.017	0.040	0.084	0.065 $\pm$ 0.012	11.254	0.002	9.6
VCS	0.067	0.139	0.100 $\pm$ 0.018	0.067	0.108	0.085 $\pm$ 0.010	11.944	0.001	7.7
HW	0.205	0.281	0.235 $\pm$ 0.020	0.185	0.265	0.218 $\pm$ 0.021			
HL	0.090	0.157	0.128 $\pm$ 0.017	0.098	0.153	0.121 $\pm$ 0.013	2.341	0.133	2.6
LA	133.55	360.21	235.76 $\pm$ 45.30	105.40	220.68	159.05 $\pm$ 31.87	47.964	< 0.0001	19.4
PP	0.028	0.052	0.039 $\pm$ 0.006	0.024	0.037	0.031 $\pm$ 0.003	39.157	< 0.0001	12.3
PS	130.57	223.77	168.91 $\pm$ 27.77	61.88	165.44	126.68 $\pm$ 23.65	33.523	< 0.0001	14.3
DAE	0.155	0.231	0.193 $\pm$ 0.019	0.147	0.212	0.175 $\pm$ 0.017		11.286	0.002
DPE	0.138	0.199	0.173 $\pm$ 0.016	0.133	0.193	0.165 $\pm$ 0.015	3.562	0.065	2.5
DAPE	0.022	0.042	0.032 $\pm$ 0.004	0.019	0.033	0.025 $\pm$ 0.004	32.043	< 0.0001	11.0

Population size-structure

A total of 275 worms were collected during this study, 246 infesting *S. plana* (241 during the seasonal monitoring, five in January 2013), 26 in *M. pellucida* (plus three outside the host, but in the same container) (Table 9). Sizes ranged from 1.8 to 3.6 mm. Small-sized worms seem to be better represented during late autumn, but also in winter, while large worms were more or less constantly present throughout the study period, except during late winter and early spring (Fig. 4).

Ripe females occurred during the whole period except in April 2012, and were always among the largest size-classes (2.2 to 3.6 mm) (Fig. 4). The highest percentages occurred in mid spring and summer, being August 2011 the single month during which the proportion ripe females vs. non-sexed adults sexes was 1:1 (Fig. 5; Table 9). The lowest percentages occurred in autumn and winter (Fig. 5; Table 9).

A total of 6,917 specimens of *S. plana* and 39 of *M. pellucida* were collected (Table 9). Length in *S. plana* ranged from 20 to 40 mm, with the exception of one infested host measuring 40.7 mm. However, bivalves with

intermediate lengths (i.e., 26–36 mm) occurred during the whole year, being always the most abundant and also the most infested ones (Fig. 6). The most balanced size class frequency distribution occurred in September 2011. In *M. pellucida*, the size-class range was restricted to 20–30 mm length, and the most infested ones were slightly smaller (20–28 mm) than in *S. plana* (Fig. 6).

Overall, there was a non-significant size correlation between *O. okupa* sp. nov. and the host *S. plana* (Pearson coefficient = 0.123, $p = 0.067$). The monthly trends were also non-significant (Pearson coefficient = -0.375 to 0.351, $p = 0.103$ to 0.818), except for a positive correlation in April 2012 (Pearson coefficient = 0.870, $p = 0.011$). Conversely, *O. okupa* sp. nov. / *M. pellucida* symbiont-host pairs (26) collected in January 2013 showed a significant, positive size correlation (Pearson coefficient = 0.400, $p = 0.021$).

Infestation characteristics

The studied population of *O. okupa* sp. nov. showed a seasonal variability in prevalence (Fig. 5; Table 9). The highest percentages occurred during late autumn-mid

Fig. 4. *Oxydromus okupa* sp. nov. Size-frequencies of the specimens hosted by *Scrobicularia plana* from April 2011 to May 2012, and by *Macomopsis pellucida* in January 2013. 2011: April, n = 16; June, n = 22; July, n = 15; August, n = 10; September, n = 17; October, n = 11; November, n = 28; December, n = 49. 2012: January, n = 23; February, n = 20; March, n = 8; April, n = 7; May, n = 15; 2013: January, n = 26.

Table 7. List of the ten most contributing measurement proportions to the intra-population similarities and inter-population dissimilarity based on the SIMPER analyses. Measurement abbreviations as in Fig. 1. Av.Value: Average value; Av.Sq.Distance: average square distance; Sq.Distance/SD: square distance divided by standard deviation; Contrib%: percentage of contribution; Cum.%: cumulative percentage of contribution.

Iberian	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%	
DAPE/HL	0.531	0.729	0.50	3.41	3.41	
DCSS/DL	0.663	0.738	0.49	3.45	6.86	
LA/HL	0.599	0.84	0.47	3.93	10.79	
DAE/DPE	0.481	0.863	0.47	4.04	14.82	
DPE/HW	-0.203	0.906	0.48	4.24	19.06	
DCSS/DL	0.515	0.913	0.49	4.27	23.33	
PS/HL	0.518	0.936	0.51	4.38	27.70	
PP/HL	0.495	0.97	0.50	4.54	32.24	
WWP/WW	-0.527	0.977	0.49	4.57	36.81	
NS/WW	-0.578	0.991	0.49	4.63	41.44	
Congolese	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%	
WL/WW	-0.176	0.175	0.47	1.58	1.58	
NS/WW	0.657	0.209	0.44	1.89	3.47	
DCSS/DL	-0.753	0.23	0.48	2.07	5.54	
LA/HL	-0.68	0.319	0.50	2.87	8.41	
WWP/WW	0.598	0.369	0.51	3.33	11.74	
PS/HL	-0.589	0.439	0.37	3.96	15.69	
PP/HL	-0.563	0.458	0.46	4.13	19.82	
DCSS/DL	-0.585	0.472	0.48	4.26	24.08	
VCS/PNCS	-4.07E-2	0.496	0.51	4.47	28.56	
DCSS/DCPS	-0.477	0.545	0.46	4.92	33.47	
Variable	Iberian Av.Value	Congolese Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
DCSS/DL	0.663	-0.753	2.93	0.96	6.38	6.38
LA/HL	0.599	-0.68	2.75	0.98	5.97	12.35
NS/WW	-0.578	0.657	2.67	0.81	5.82	18.17
DAPE/HL	0.531	-0.604	2.6	0.84	5.65	23.82
WWP/WW	-0.527	0.598	2.56	0.84	5.56	29.38
PS/HL	0.518	-0.589	2.54	0.74	5.53	34.91
DCSS/DL	0.515	-0.585	2.54	0.77	5.52	40.43
PP/HL	0.495	-0.563	2.49	0.91	5.41	45.84
DAE/DPE	0.481	-0.546	2.47	0.87	5.38	51.22
DCSS/DCPS	0.42	-0.477	2.33	0.81	5.07	56.28

winter. The highest peak was in December 2011 (i.e. 19.2%), in coincidence with the highest number of symbionts (Table 9). Intermediate prevalences occurred in November 2011 (i.e. 7.2%), January 2012 (i.e. 8.2%) and February 2012 (i.e. 6.1%). The remaining prevalences were always < 5% and the lowest one occurred in April 2011 (i.e. 1.6%). The intensity of the infestation was always a single worm per host. In the case of the specimens associated with *M. pellucida*, the prevalence was 66.7% (Table 9), reaching 89.3% in hosts > 20 mm long (Fig. 6).

There was a non-significant correlation between the percentage of ripe females and prevalence in *S. plana* along the studied period (Pearson coefficient = -0.407, $p = 0.168$). However, the percentage of ripe females was, on average, significantly higher (one-way ANOVA, $F = 9.253$, $p = 0.011$) in the warm than in the cold period, reaching $31.0 \pm 4.8\%$ and $7.5 \pm 6.1\%$, respectively, while the prevalence showed exactly the contrary pattern, $3.2 \pm 1.3\%$ vs. $8.9 \pm 1.7\%$ (one-way ANOVA, $F = 7.066$, $p = 0.022$) (Fig. 5).

Table 8. Comparative table of the morphometric measurement proportions in the Iberian and Congolese populations. Differences expressed as percentages, with bold characters indicating significant differences (according to Benjamini and Hochberg, 1995) that may be higher (normal text) or lower (italics) in the Iberian than in the Congolese population. Measurement abbreviations as in Fig. 1. Min: minimum; Max: maximum; Mean: average $\pm$ standard deviation; F: Fisher's F index; p: significance level.

SD	Iberian Population			Congolese Population			ANOVA		Difference	
	Min	Max	Mean	Min	Max	Mean	F	p	%	
WL/WW	4.129	10.449	7.696 $\pm$ 1.903	6.154	8.658	7.214 $\pm$ 0.609	1.379	0.246	3.2	
NS/WW	0.014	0.027	0.021 $\pm$ 0.004	0.023	0.031	0.026 $\pm$ 0.002	30.385	< 0.0001		9.6
WWP/WW	0.284	0.492	0.399 $\pm$ 0.054	0.403	0.578	0.462 $\pm$ 0.041	21.926	< 0.0001		7.3
DCPL/DLL	0.125	0.314	0.201 $\pm$ 0.053	0.100	0.253	0.175 $\pm$ 0.041	3.809	0.057	6.9	
DCSL/DLL	1.309	3.038	2.061 $\pm$ 0.449	0.961	2.211	1.513 $\pm$ 0.316	24.871	< 0.0001	15.3	
DCSL/DCPL	5.501	17.797	10.658 $\pm$ 2.530	6.814	13.446	8.886 $\pm$ 1.915	7.806	0.007	9.1	
VCL/PNCL	0.709	2.477	1.281 $\pm$ 0.361	0.949	2.049	1.326 $\pm$ 0.276	0.246	0.622		1.7
DCPS/DLS	0.124	0.324	0.193 $\pm$ 0.057	0.092	0.251	0.160 $\pm$ 0.041	5.464	0.024	9.3	
DCSS/DLS	1.220	2.750	1.920 $\pm$ 0.406	0.826	1.716	1.226 $\pm$ 0.229	55.281	< 0.0001	22.1	
DCSS/DCPS	5.920	16.985	10.520 $\pm$ 2.915	4.934	12.884	8.013 $\pm$ 2.011		12.531	0.001	13.5
VCS/PNCS	0.897	3.332	1.350 $\pm$ 0.504	0.888	2.673	1.377 $\pm$ 0.387	0.046	0.831		1.0
HL/HW	0.420	0.642	0.545 $\pm$ 0.059	0.430	0.680	0.559 $\pm$ 0.057	0.727	0.398		1.3
LA/HL	0.362	0.967	0.721 $\pm$ 0.145	0.400	0.690	0.518 $\pm$ 0.092	34.956	< 0.0001	16.4	
PP/HL	0.215	0.395	0.309 $\pm$ 0.049	0.211	0.341	0.254 $\pm$ 0.032	21.893	< 0.0001	9.8	
PS/HL	0.356	0.698	0.517 $\pm$ 0.095	0.204	0.533	0.413 $\pm$ 0.066	20.384	< 0.0001	11.2	
PS/PP	1.171	2.641	1.707 $\pm$ 0.385	0.751	2.206	1.651 $\pm$ 0.319	0.311	0.580	1.7	
DAE/HW	0.741	0.924	0.821 $\pm$ 0.042	0.725	0.871	0.805 $\pm$ 0.035	2.179	0.146	1.0	
DPE/HW	0.660	0.832	0.738 $\pm$ 0.042	0.677	0.852	0.758 $\pm$ 0.043	2.744	0.104		1.3
DAPE/HL	0.195	0.303	0.250 $\pm$ 0.031	0.155	0.269	0.211 $\pm$ 0.028	22.185	< 0.0001	8.6	
DAE/DPE	1.021	1.198	1.113 $\pm$ 0.046	1.004	1.148	1.063 $\pm$ 0.042	16.107	0.000	2.3	

Year	Month	Hosts		% Ripe Females	Prevalence	Host species
		Total	Infested			
2011	April	1000	16	37.5	1.6	<i>S. plana</i>
	June	468	22	40.9	4.7	
	July	469	15	40.0	3.2	
	August	370	10	50.0	2.7	
	September	370	17	41.2	4.6	
	October	306	11	9.1	3.6	
	November	389	28	10.7	7.2	
	December	255	49	8.2	19.2	
2012	January	280	23	4.3	8.2	
	February	328	20	5.0	6.1	
	March	320	8	25.0	2.5	
	April	219	7	0.0	3.2	
	May	469	15	13.3	3.2	
2013	January	39	26	2.9	66.7	<i>P. cumana</i>

Table 9. *Oxydromus okupa* sp. nov. Synthesis of the monthly captures, total and infested number of hosts, percentage of ripe females, and prevalence during the study period.

Discussion

Morphometry and taxonomy

Molecular analyses currently tend to displace morphometry in studies trying to discriminate within

closely related morphotypes for taxonomic purposes (Blaxter, 2004; Hebert and Gregory, 2005; Godfray, 2007). However, most taxonomists agree that combining the different approaches, applying the so-called Integrative Taxonomy, is the most effective strategy to build a stable and robust taxonomy (Will

Fig. 5. *Oxydromus okupa* sp. nov. Monthly prevalence of ripe females vs. total prevalence in the infested *Scrobicularia plana*.

et al., 2005; Padial *et al.*, 2010). Hence, the importance of morphology must not be forgotten in the genomics era (Giribert, 2015). Moreover, it is not always possible to obtain adequate material for genetic studies because the used preservation methods are destructive, while there is no fresh material available. In such cases morphometry may be the only approach allowing to identify distinguishing characters within species complexes.

Among polychaetes, many taxonomically robust morphological characters may be defined by measurements and/or proportions, some of them being size-dependent (Ben-Eliahu, 1987; Fauchald, 1991; Sigvaldadóttir and Mackie, 1993). This approach has been used independently of molecular analyses to successfully resolve the taxonomy of several sibling species complexes, often leading to the description of new species (Orrhage and Sundberg, 1990; Fauchald, 1991; Blake, 2000; Koh and Bhaud, 2003; Koh *et al.*, 2003; Martin *et al.*, 2003, 2006, 2009; Ford and Hutchings, 2005; Garraffoni and de Garcia Camargo, 2006; Glasby and Glasby, 2006; Lattig *et al.*, 2007; Hernández-Alcántara and Solís-Weiss, 2014; Coutinho *et al.*, 2015). However, Ford and Hutchings (2005) were the first to consider the use of the statistical dissimilarities derived from the SIMPER routine of the PRIMER software (Clarke and Warwick, 2001; Clarke and Gorley, 2006), based on a matrix of morphometric measurements, as a robust support to distinguish between morphologically close species. Accordingly, they described three new species whose average dissimilarities ranged between 11–19%, later finding morphological evidences supporting the

erection of the new species. Our morphometric approach, in turn, clearly discriminates the two populations of “*O. humesi*” which, despite their close morphologies, showed average dissimilarities ranging from 30 to 36% in the SIMPER, thus almost three times higher than the Australian species of *Owenia*. A detailed comparison of character variability, particularly those that were responsible for the intra-population similarity and the inter-population dissimilarity, led to a reliable way to solve this question. There were significant differences in size range between the Iberian and the Congolese populations (more restricted in the later, leading us to find less size-correlated parameters in Congolese worms). Our approach to compare the two populations led to evident and consistent differences in the appendage measurements and relative proportions (Tables 6, 8) that are considered as robust enough to formally describe the Iberian specimens as the new species *O. okupa* sp. nov. In addition, we further validate these differences by means of discriminant analyses, whose respective discriminant functions were able to identify the members of the two species with a reliability of 96 to 100% in all tests.

Infestation characteristics

Host/symbiont size relationships. Positive host/symbiont size relationships may either indicate 1) active size segregation behaviour, as reported both for the symbiotic coral dwelling crab *Trapezia bidentata* (Forskål, 1775) (Adams *et al.*, 1985) and the fish *Gobiodon histrio* (Valenciennes, 1837) (Hobbs and Munday, 2004), which seem to be able to migrate from colony to colony to choose one of an appropriate size, or 2) parallel growth of hosts and symbionts, as reported both for the nemertean *Malacobdella grossa* (Müller, 1776), hosted by *Arctica islandica* (Linnaeus, 1767) (Sundet and Jobling, 1985) and the pontonin shrimp *Anchistus custos* (Forskål, 1775), hosted by the bivalve *Pinna bicolor* Gmelin, 1791 (Britayev and Fahrutdinov, 1994). In turn, the absence of host/symbiont size correlation could be caused by symbionts that either colonize the hosts during a single phase of their life-history (e.g. juveniles, adults), that are highly mobile (thus, colonizing the available host independently of their size), or growth faster than the host, even if the infestation starts with juveniles colonizing small-sized hosts (Britayev *et al.*, 2007).

Among symbiotic polychaetes, positive host/symbiont size-relationships are not common (Martin and Britayev, 1998) and, when occurring, they may be modified by differential behaviour linked to, for instance,

Fig. 6. Size-frequencies of the total vs. infested *Scrobicularia plana* from April 2011 to May 2012 and *Macomopsis pelucida* in January 2013 (n = 39). Monthly total number of individuals of *S. plana*: 2011: April, n = 300; June, n = 200; July, n = 94; August, n = 100; September, n = 108; October, n = 99; November, n = 99; December, n = 124. 2012: January, n = 10; February, n = 98; March, n = 150; April, n = 102; May, n = 100.

reproductive activities. This seems to be the case of *Branchiopolynoe seepensis* Pettibone, 1986, an obligate symbiont of deep-sea hydrothermal vent mytilids of the genus *Bathymodiolus*, whose females have a longer life span than males and remain inside the same host along their whole life, while males leave their original hosts for mating and then colonize the available hosts independently of their size. Consequently, positive host/symbiont size correlations occur only for females and for non-mature males (Britayev *et al.*, 2007). In most known cases, however, the relationship between size structure of symbiotic polychaetes and their hosts is unclear (Martin *et al.*, 1991, 1992; Emson *et al.*, 1993; Rozbaczylo and Cañete, 1993; Britayev and Zamyshliak, 1996) so that the life histories of the former have been considered to be independent of that of the latter, with the hosts tending to live longer and commonly hosting successive symbionts (Martin and Britayev, 1998).

The absence of host/symbiont size-relationships previously reported for the intertidal population of *O. okupa* sp. nov. (Martin *et al.*, 2012) was here confirmed by the absence of significant correlations found along the whole study period. The only exception was April 2012, which we consider as a spurious relationship due to the low number of collected worms ($n < 10$) (Fig. 4; Table 9). However, other months with a similar number of individuals (e.g. $n = 8 - 11$ in March, October, and August) (Fig. 4; Table 9) showed non-significant relationships. These results support Martin *et al.* (2012) in that the worms do not grow together with their hosts in their intertidal environment. In turn, *O. okupa* sp. nov. was reported to infest specimens of *S. plana* > 20 mm in shell length and to be more frequent in intermediate-sized shells, 26–36 mm long (Martin *et al.*, 2012), which has also been confirmed along the whole period of study. Limiting host sizes have been reported for the starfish *Asterias rathbuni* (Verrill, 1909) in Vostok Bay (Sea of Japan), whose specimens with disc radii lower than 35 mm were not inhabited by *Arctonoe vittata* (Grube, 1855), and those with radii up to 90 mm harboured one single symbiont, whereas the largest starfishes could host up to four polychaetes (Britayev *et al.*, 1989). Another example occurs in Chile, where the scale-worm *Harmothoe commensalis* Rozbaczylo and Cañete, 1993 did not occur in specimens of the clam *Gari solida* (Gray, 1828) < 60 mm in shell length (Rozbaczylo and Cañete, 1993). The size-limit for *S. plana* as a host of *O. okupa* sp. nov., also implied that there were no small juveniles infesting small-sized hosts, which allows us to discard the fast-growing hypothesis for this new species.

On the other hand, the infested specimens of *M. pellucida* collected in a subtidal environment during January 2013 included a considerable amount of small-sized hosts (Fig. 6). Moreover, there was a significant positive size correlation between hosts and symbionts. This relationship may be caused either by the symbiont selecting the host to be infested according to the appropriateness of its size, or by host/symbiont parallel growth, which cannot be assessed in light of our present data. Despite the low number of collected bivalves, the existing size relationship and the very high infestation rates (Table 9), suggest together that *M. pellucida* and the subtidal environment could be the preferred host and habitat for *O. okupa* sp. nov., with the symbiont selecting the most suitable hosts when conditions are optimal.

Prevalence of the infestation. The prevalence of commensal polychaetes is highly variable and has been considered as a species-specific characteristic. However, a commensal species may also show different prevalences, which may vary according to bathymetric, spatial and temporal (i.e. intra- and inter-annual) patterns (Martin and Britayev, 1998). Studies including temporal trends are, however, scarce. With the exception of the impressive fidelity of the year-to-year counts of a regular seasonal trend reported for the infestation prevalence of *Ophiocoma echinata* (Lamarck, 1816) by *Branchiosyllis exilis* (Gravier, 1900) in Panamá (Hendler and Meyer, 1982), the only known data on year-to-year variability for a symbiotic polychaete are probably those on *Arctonoe vittata* (Grube, 1855). In Vostok Bay, this worm infests the starfish *Asterias amurensis* Lütken, 1871 with a progressive increase in prevalence from 0% in 1975–76 to 8.4% in 1978 and to 79.1% in 1980 (Britayev, 1991). Based on our study period, *O. okupa* sp. nov. seems to show a seasonal trend in prevalence, with the highest percentages occurring during the coldest seasons, i.e. from late autumn to late winter (Fig. 5; Table 9). The present dataset only covers a single year and therefore inter-annual regularities cannot be inferred.

Theoretically, we may expect an influence of the host's population structure on that of the symbiont (Martin & Britayev, 1998). A positive relationship between host density and infestation characteristics was reported for *A. vittata* and its host starfish *A. rathbuni* in Vostok Bay (Britayev *et al.*, 1989). The highest prevalence, mean intensity and abundance depended upon host's density, which could be caused by the accumulation of a chemically-mediated host-cue more effectively attracting the settling symbionts. High host densities may also reduce the influence of external factors. For

instance, commensals associated with less abundant host populations may experience a more relevant decrease in fitness than those harboured by dense host populations (Martin and Britayev, 1998).

Among the few relationships with known data, high infestation indexes tend to be positively correlated with the availability of large and numerous hosts to be occupied by the symbionts (Martin and Britayev, 1998). In the present case, the overall low prevalence would lead to the expectation of a low number of *S. plana* > 20 mm long in the study area. Conversely, *S. plana* was very abundant independently of size (Subida *et al.*, 2011; Drake *et al.*, 2014) and so numerous adequate hosts were available through the whole study period (Fig. 6; Table 9). Therefore, the prevalence seems not to be connected with the host availability. In turn, this low prevalence could be related to the extreme daily changes in temperature and/or the alternating long desiccation/immersion periods that characterize the intertidal environment, as well as with biota disturbance due to bait digging of this zone at Río San Pedro (Carvalho *et al.*, 2013). The high prevalence in the subtidal population of *M. pellucida* (i.e. higher than 85% in specimens > 20 mm long), seems to confirm this hypothesis, with the permanent immersion in the subtidal environment favouring the presence of the symbiont.

The fact that no specimens of *S. plana* < 18 mm in shell length were found during the annual cycle, is in agreement with all previous data obtained in punctual samplings (Martin *et al.* 2012, 2015). The present approach did not clarify this peculiarity of the studied bivalve population. However, the high density of specimens restricted to the narrow intertidal area at Río San Pedro led to postulate the existence of a negative interaction between the established population and the settling larvae, which could be actively ingested by their conspecific adults during their normal filter/suspension feeding activities (i.e. passive cannibalism) (Cargnin-Ferreira, 2005; Santos *et al.* 2011).

The association of a symbiont with various hosts in the same locality could affect the prevalence, which strongly depends on its level of affinity for the different hosts. Although the symbiotic population of *O. okupa* sp. nov. at Río San Pedro only infested *S. plana*, this factor could not be eventually discarded due to the presence of the nearby subtidal population of *M. pellucida* at the opening of Río San Pedro.

Infestation intensity. The infestation intensity of symbiont polychaetes has been more widely reported than the prevalence. It may range from one to hundreds of sym-

bionts per host, but is clearly dominated by the association of a single symbiont per host either due to the usually low symbiont densities or to the influence of an intraspecific aggressive behaviour (Martin and Britayev, 1998). Like the prevalence, intensity may also oscillate within the same population due to seasonal changes in relative abundances, linked or not to reproduction and recruitment events. A common situation for symbionts with 1:1 regular distributions like *O. okupa* sp. nov., is that adults may occasionally share the host with one to several juveniles, as previously reported for polychaete species of the genera *Acholoe*, *Adyte* or *Branchiosyllis*, among others (Martin and Britayev, 1998).

In the case of *O. okupa* sp. nov., Martin *et al.* (2012, 2015) reported single findings of one male and one female and one male, one female and one small worm, likely a juvenile sharing the same host, while the constant regular distribution of one single symbiont per host was attributed to intraspecific aggressive behaviour. There are several cases of symbiotic polychaetes with male and female couples living together associated with the same host, such as the Mediterranean fish parasite *Ichthyotomus sanguinarius* Eising, 1906 (Eising, 1906; Culurgioni *et al.*, 2006) or the deep-sea hexactinellid sponge symbiont *Robertianella synophthalma* McIntosh, 1885, reported as *Harmothoe hyalone-mae* in Martin *et al.* (1992). However, in both cases, most hosts harboured couples, while in *O. okupa* sp. nov. couples seemed to be the exception.

Life-cycle

Little is known about the life cycle of most commensal polychaetes and, when known usually does not differ much from that of their free-living relatives. Thus, it is expected to find planktonic larvae (responsible of dispersal and colonization) and benthic adults (with a somewhat reduced mobility) with the single main difference that the symbiotic mode of life replaces the free-living one during the benthic phase (Martin and Britayev, 1998). Larval settlement may occur on the bottom, being then followed by a juvenile migration towards the respective hosts (Davenport and Boolootian, 1966). However, it seems more likely that chemically mediated cues (either generated by the host or by the own symbiotic adults) driving larval settlement could be the most widespread behaviour among symbiotic polychaetes (parasites included) (Martin and Britayev, 1998).

As it occurs for the free-living species, this basic life-cycle scheme may vary in many ways as a result of the adaptation to a symbiotic mode of life. For instance,

it can be simplified by reducing (or even eliminating) the free-living pelagic stage or become more complex by having one or more intermediate hosts, which are occupied when the preferred ones are not available or because they have more room to host several juveniles during growing period (Martin and Britayev, 1998). Herewith, intraspecific competition and aggression may play a major role in the associated relocation processes. In the case of *O. okupa* sp. nov., the only information known on the juveniles is that they have not been found inside *S. plana*, neither free-living, which has been confirmed during the studied period.

The highest number of small worms found inside *S. plana* occurred in late autumn, which could be considered as an indication of new symbiont's recruitment into the host bivalves (after the population being more actively reproducing during summer). In turn, large adults tended to disappear around mid-winter. This may suggest that the life span of *O. okupa* sp. nov. may be of one year, with the adults dying after reproducing. However, sampling during successive years would be required to confirm this hypothesis, as well as to assess the regularity of the recruitment events.

The life cycle of *O. okupa* sp. nov. may be limited by the tidal regime characteristic of its intertidal habitat, while the highly abundant population living in *M. pellucida* seems to indicate that recruitment may occur mainly in the more favourable subtidal conditions. Thus, we propose a possible scenario in which the symbionts mainly inhabit certain areas of the Bay not submitted to periodical desiccation by tides, living in association with *M. pellucida* (but we cannot discard other possible unknown hosts), then reaching intertidal areas such as those at Río San Pedro either as larvae through tidal currents or by adult migration. Therefore, the intertidal area here studied could be at the limit of the ecological distribution of this bivalve endosymbiont in the Cádiz Bay region.

The absence of juveniles inside the studied hosts suggests that this phase may be free-living and that the colonization of *S. plana* occurs during the benthic phase of the life cycle of *O. okupa* sp. nov., whose adults are able to move up along Río San Pedro with tides. However, neither juveniles, nor adults have been found in the sediments surrounding the studied areas (P. Drake, per. observ.), likely because they may be quite rare.

The possible life cycle of *O. okupa* sp. nov. may thus consist of 1) a planktonic larval phase settling on soft bottoms, 2) free-living juveniles, and 3) adults able to select (whenever possible) and enter the hosts at a given size (i.e., >1.6 mm wide according the present results). If so, phase 3 may involve thigmotaxis and the highly

specific host entering behaviour described by Martin et al. (2015). In fact, a comparable life cycle was described for the polychaete *Neanthes fucata* (Savigny in Lamarck, 1818) in hermit crabs, by Gilpin-Brown (1969) off Plymouth. The planktotrophic larvae of this nereidid settle directly on soft bottoms. The juveniles live in tubes for several months feeding on detritus and small benthic animals exactly as many of their free-living relatives. Then, 4-month old worms start to develop the ability to recognize the presence of potential hosts by the substratum vibrations produced by the hermit crab legs bouncing on the sediment surface, which triggers a characteristic host-entering behaviour that allows the worms to crawl on the hermit crab shell following the shell spirals by thigmotaxis (Gilpin-Brown, 1969). This complex life cycle uses different mechanisms that characterize the free-living nereidids (such as thigmotaxis or mucus production) as specific adaptations to the commensal mode of life, while the worm itself has no relevant morphological adaptations. Therefore, in addition to the similarity of the hypothesized life cycle of *O. okupa* sp. nov., both species also share the lack of evident morphological adaptations (maybe except for the reduction of the central antennae in the hesionid, whose significance in terms of adaptation to the symbiotic mode of life remains unclear) and an equivalent, highly specific host entering behaviour.

The mechanism of host-recognition behaviour in *O. okupa* sp. nov. is currently unknown, and the presence of a host-factor has not been demonstrated (Martin et al., 2012, 2015). However, it is well known that the bivalves hosting *O. okupa* sp. nov. may alternate between direct water filtration and deposit feeding by tapping on the sediment surface with their inhalant siphons. Thus, we may hypothesize that the symbiont, like *N. fucata*, may recognize the presence of a potential host by the movements of the inhalant siphon. Despite aforementioned similarities between the known hosts of *O. okupa* sp. nov., there is no direct evidence of the symbiont entering into *M. pellucida* (or any other potential host) in a similar way as into *S. plana*.

Relationships between life-cycle and infestation characteristics

The infestation in *O. okupa* sp. nov., seemed to be connected with the reproduction. The months with lower prevalence (i.e., mid spring and summer) showed a markedly higher percentage of ripe females (>30%) (Fig. 4, 5; Table 9), reaching a 50% in August 2011, while the coldest month (i.e. October 2011 to February

2012) showed a high prevalence coupled with low percentages of ripe females (Fig. 4, 5; Table 9). The marked seasonal pattern found in the population of *O. pugettensis* (Johnson, 1901) infesting the starfish *Patiria miniata* (Brandt, 1835) at Dana Point (California) seemed also to be connected with the commensal reproductive dynamics (Lande and Reish, 1968). This species reached the highest ($\geq 80\%$) and lowest ($\leq 30\%$) prevalence in November–December and prior to the decrease of water temperature in summer, respectively. Its abundance was maximum in winter (2–3 worms per host) and minimum at mid-summer (< 0.5 worms per host).

Despite sampling on different years is certainly required to infer regularities in the relationships between prevalence and life cycle in *O. okupa* sp. nov., these observations suggests that ripe females may have a reduced mobility (i.e., they tend to remain inside the host during their whole life), whereas males could be more mobile and thus leave their host, likely to increase fertilization success. Taking into account that *O. okupa* sp. nov. has a 1:1 regular distribution, the higher mobility attributed to males may contribute to an increase fertilization success by increasing the possibility of male/female partnership. Two possible mechanisms may explain this: 1) males may directly enter a host occupied by a ripe female, or 2) males may approach a host when females are releasing their sexual products. In both cases, we suggest that chemical cues may be involved. A similar behaviour was previously reported for *B. seepensis*, and, as mentioned above, also contributed to explain the lack of host-bivalve size relationships for adult males (Britayev *et al.*, 2007). A similar situation was also reported for *Haplosyllides floridana* Augener, 1922, whose male stolons were never found inside the host sponge *Neofibularia nolitangere* (Duchassaing & Michelotti, 1864) and, conversely, were found free-swimming in the water column (Martin *et al.*, 2009). Accordingly, the highest prevalence in the studied population of *O. okupa* sp. nov. occurred when both males and females occupied their respective hosts.

Despite this general pattern of reproduction vs. prevalence, *O. okupa* sp. nov. seems to reproduce actively during the whole year, but with an increasing effort during spring–summer and a higher intensity in summer. The absence of ripe females in April 2012 contrasted with the almost 40% found in 2011. However, ripe females occurred both before and after April 2012, allowing us to suggest that its absence in April 2012 could have been biased by the low number of symbionts found in this month (Fig. 4; Table 9). Persistent high temperatures can stimulate oocyte growth, subsequently caus-

ing the advancement of reproductive period in polychaetes, as reported for the Mediterranean populations of *Eupolyornia nebulosa* (Montagu, 1819) (Cha *et al.*, 1997). However, our data does not allow assessing whether environmental constraints such as differences in temperature could affect the reproductive cycle of *O. okupa* sp. nov. during the studied period.

Current knowledge on hesionid symbionts

Thirty out of 170 currently known hesionid species live as commensals of other invertebrates (Martin and Britayev, 1998; Miller and Wolf, 2008; De Assis *et al.*, 2012; Martin *et al.*, 2012, 2015; Britayev *et al.*, 2013; Chim *et al.*, 2013), representing around 18% of all the known hesionid species and about 6% of the known symbiotic polychaetes (Table 10). Symbiosis seems to be restricted to the clade Ophiodrominae, which includes the genus *Oxydromus*. It has multiple origins within the family Hesionidae because *Oxydromus* and *Gyptis*, two of its most representative genera with symbiotic species, are not closely related phylogenetically (Ruta, *et al.*, 2007). Moreover, both are species-rich genera and there is no evidence indicating whether commensalism arose once or multiple times within each of them.

Commensal hesionids are involved in about 65 different associations (Table 10). Except for the polyxeneous species of *Oxydromus*, such as for *O. flexuosus* (Delle Chiaje, 1827) and *O. pugettensis* (Johnson, 1901) with up to 10 and 11 hosts, respectively, most symbiotic hesionids are monoxeneous, occurring in only one (15 species) and two (6 species) hosts (Table 10). Hesionid hosts include species from very different taxonomic groups, a variety among symbiotic polychaetes only comparable to that of polynoids (Martin and Britayev, 1998). The most common hosts are, however, echinoderms (particularly starfishes and sea urchins) and polychaetes (Table 10).

Only one genus, *Oxydromus*, includes symbiotic species living in association with bivalves. These are *O. pugettensis*, *O. humesi* and *O. okupa* sp. nov. Although nothing is known on the relationships between *O. pugettensis* and its bivalve host, the polychaete seems to be able to detect at a certain distance the presence of at least two of its host starfishes, *P. miniata* and *Luidia foliolata* (Grube, 1866) (Davenport *et al.*, 1960). Also there are some indications of mutualistic behaviour in their relationships with one of its echinoid hosts, the sand dollar *Clypeaster humilis* (Leske, 1778) (Storch and Niggemann, 1967). The existing analyses of the behaviour of *O. okupa* sp. nov. in experimental condi-

Table 10. List of the currently known symbiotic species within the family Hesionidae (*sensu lato*).

SPECIES	AUTHORITY	HOST GROUP	HOST SPECIES	AUTHORITY	SOURCE
<i>Gyptis ophiocomae</i>	Storch & Niggemann, 1967	Ophiuroid	<i>Ophiocoma scolopendrina</i>	(Lamarck, 1816)	Storch and Niggemann, 1967
<i>Gyptis vittata</i>	Webster & Benedict, 1887	Polychaete	<i>Notomastus lobatus</i>	Hartman, 1947	Gardiner, 1976; Pettibone, 1963
<i>Hesione picta</i>	Müller in Grube, 1858	Ophiuroid	<i>Ophionereis reticulata</i>	(Say, 1825)	De Assis, Bezerra, Brito, Gondim and Christoffersen, 2012
<i>Hesionella mccullochae</i>	Hartman, 1939	Polychaete	<i>Lumbrineris brevicirra</i>	(Schmarda, 1861)	Hartman, 1939
<i>Leocrates chinensis</i>	Kinberg, 1866	Cnidarian	Corals		Pettibone, 1970
<i>Leocrates clapedii</i>	(Costa in Claparède, 1868)	Cnidarian	Corals		Pettibone, 1970
<i>Microphthalmus aberrans</i>	(Webster & Benedict, 1887)	Polychaete	<i>Lysilla alba</i>	Webster, 1879	Pettibone, 1963
		Polychaete	<i>Enoplobranchus sanguineus</i>	(Verrill, 1873)	Pettibone, 1963
<i>Microphthalmus hamosus</i>	Westheide, 1982	Sipunculan	<i>Sipunculus (Sipunculus) nudus</i>	Linnaeus, 1766	Westheide, 1982
<i>Microphthalmus szcelkowi</i>	Metschnikow, 1865	Polychaete	<i>Arenicola marina</i>	(Linnaeus, 1766)	Reise and Ax, 1979
<i>Oxydromus angustifrons</i>	(Grube, 1878)	Asteroid	<i>Pentaceraster multispinus</i>	(von Martens, 1866)	Jones, 1964
		Echinoid	<i>Salmacis sphaeroides</i>	(Linnaeus, 1758)	Chim et al., 2013
		Echinoid	<i>Temnopleurus toreumaticus</i>	(Klein, 1778)	Chim et al., 2013
		Echinoid	Unknown sand dollars		Mohammad, 1971
<i>Oxydromus bunbuku</i>	(Uchida, 2004)	Echinoid	<i>Brissus agassizii</i>	Döderlein, 1885	Uchida, 2004
		Echinoid	<i>Brissus latecarinatus</i>	(Leske, 1778)	Uchida, 2004
<i>Oxydromus flexuosus</i>	(Delle Chiaje, 1827)	Asteroid	<i>Astropecten aranciatus</i>	(Linnaeus, 1758)	Barel and Kramers, 1977
		Asteroid	<i>Astropecten bispinosus</i>	(Otto, 1823)	Barel and Kramers, 1977
		Asteroid	<i>Astropecten indicus</i>	Döderlein, 1888	Barel and Kramers, 1977
		Asteroid	<i>Astropecten irregularis</i>	(Pennant, 1777)	Barel and Kramers, 1977
		Asteroid	<i>Luidia ciliaris</i>	(Philippi, 1837)	Barel and Kramers, 1977
		Hemichordata	<i>Balanoglossus</i> sp.		Clark, 1956
		Echiuroid	<i>Maxmuelleria lankesteri</i>	(Herdman, 1897)	Anker et al., 2005
		Holothuroid	<i>Leptosynapta</i> sp.		Barel and Kramers, 1977
		Polychaete	<i>Neoamphitrite edwardsi</i>	(Quatrefages, 1866)	Barel and Kramers, 1977
		Polychaete	<i>Euclymene lombricoides</i>	(Quatrefages, 1866)	Barel and Kramers, 1977
<i>Oxydromus hermanni</i>	(Giard, 1882)	Hemichordata	<i>Balanoglossus robinii</i>	Giard, 1882	Giard, 1882
		Hemichordata	<i>Balanoglossus salmonetus</i>	Giard, 1882	Giard, 1882
<i>Oxydromus humesi</i>	(Pettibone, 1961)	Bivalve	<i>Tellina nimpalis</i>	Lamarck, 1818	Martin et al., 2012; Martin et al., 2015; Pettibone, 1961
<i>Oxydromus okupa</i> sp. nov.		Bivalve	<i>Scrobicularia plana</i>	(Da Costa, 1778)	Martin et al., 2012; Martin et al., 2015, this paper
		Bivalve	<i>Macoma cumana</i>	(Costa, 1830)	Martin et al., 2012; Martin et al., 2015, this paper
<i>Oxydromus obscurus</i>	(Verrill, 1873)	Polychaete	<i>Lysilla alba</i>	Webster, 1879	Pettibone, 1963
		Echinoid	<i>Lytechinus</i> sp.		Hartman, 1951
		Holothuroid	<i>Thyone</i> sp.		Pettibone, 1963

Table 10. cont.

SPECIES	AUTHORITY	HOST GROUP	HOST SPECIES	AUTHORITY	SOURCE
<i>Oxydromus pallidus</i>	Claparède, 1864	Echiuroid	<i>Lissomyema exilii</i>	(F. Müller in Lampert, 1883)	Anker et al., 2005
<i>Oxydromus pugettiensis</i>	(Johnson, 1901)	Asteroid	<i>Patiria miniata</i>	(Brandt, 1835)	Bartel and Davenport, 1956; Davenport <i>et al.</i> , 1960; Lande and Reish, 1968; Ricketts <i>et al.</i> , 1985
		Asteroid	<i>Luidia foliolata</i>	(Grube, 1866)	Davenport <i>et al.</i> , 1960
		Asteroid	<i>Luidia magnifica</i>	Fisher, 1906	Storch and Rosito, 1981
		Asteroid	<i>Pteraster tesselatus</i>	Ives, 1888	Storch and Niggemann, 1967
		Asteroid	<i>Pentacaster cumingi</i>	(Gray, 1840)	Steinbeck and Ricketts, 1941
		Asteroid	<i>Pisaster ochraceus</i>	(Brandt, 1835)	Davenport and Hickok, 1957; Hickok and Davenport, 1957
		Asteroid	<i>Luidia foliolata</i>		Hilbig, 1994; Stewart, 1970
		Holothuroid	<i>Protankyra bidentata</i>	(Woodward & Barrett, 1858)	Okuda, 1936
		Decapod	<i>Eupagurus</i> sp.		Hickok and Davenport, 1957
		Gastropod	<i>Aletes</i> sp.		Storch and Niggemann, 1967
		Bivalve	<i>Chama</i> sp.		Storch and Niggemann, 1967
<i>Oxydromus</i> sp.		Echinoid	<i>Linopneustes longispinus</i>	(A. Agassiz, 1878)	Emson, <i>et al.</i> , 1993; Miller and Wolf, 2008
<i>Oxydromus</i> sp. 1		Asteroid	<i>Luidia maculata</i>	Müller & Troschel, 1842	Britayev and Antokhina, 2012
		Asteroid	<i>Archaster angulatus</i>	Müller & Troschel, 1842	Britayev and Antokhina, 2012
<i>Oxydromus</i> sp. 2		Asteroid	<i>Luidia maculata</i>	Müller & Troschel, 1842	Britayev and Antokhina, 2012
		Asteroid	<i>Archaster angulatus</i>	Müller & Troschel, 1842	Britayev and Antokhina, 2012
		Asteroid	<i>Archaster typicus</i>	Müller & Troschel, 1842	Britayev and Antokhina, 2012
<i>Oxydromus</i> sp. 3		Echinoid	<i>Clypeaster</i> cf. <i>reticulatus</i>	(Linnaeus, 1758)	Britayev and Antokhina, 2012
<i>Oxydromus</i> sp. 4		Echinoid	<i>Salmacis bicolor</i>	L. Agassiz in L. Agassiz & Desor, 1846	Britayev and Antokhina, 2012; Britayev <i>et al.</i> , 2013
		Echinoid	<i>Rhynobrisus hemiasteroides</i>	A. Agassiz, 1879	Britayev and Antokhina, 2012
<i>Oxydromus spinapandens</i>	(Storch & Niggemann, 1967)	Echinoid	<i>Clypeaster humilis</i>	(Klein, 1778)	Storch and Niggemann, 1967
<i>Parahesione luteola</i>	(Webster, 1879a)	Decapod	<i>Upogebia affinis</i>	(Say, 1818)	Pettibone, 1956
<i>Parahesione</i> sp.		Decapod	<i>Upogebia</i> sp.		Britayev and Antokhina, 2012
<i>Podarkeopsis brevipalpa</i>	(Hartmann-Schröder, 1959)	Polychaete	<i>Glycera robusta</i>	Ehlers, 1868	Gardiner, 1976
		Holothuroid	<i>Leptosynapta tenuis</i>	(Ayres, 1851)	Gardiner, 1976
<i>Struwela noodti</i>	Hartmann-Schröder, 1959	Echinoid	<i>Mellita longifissa</i>	Michelin, 1858	Hartmann-Schröder, 1959
<i>Struwela</i> sp.		Echinoid	<i>Encope grandis</i>	L. Agassiz, 1841	Campos <i>et al.</i> , 2009
		Echinoid	<i>Encope micropora</i>	L. Agassiz, 1841	Campos <i>et al.</i> , 2009
		Echinoid	<i>Mellita granti</i>	Mortensen, 1948	Campos <i>et al.</i> , 2009
		Echinoid	<i>Mellita longifissa</i>	Michelin, 1858	Campos <i>et al.</i> , 2009

tions do not prove the existence of a host-factor in its relationships with *S. plana*, like in the case of *O. pugettensis* and its host starfishes. In turn, the species shows an elaborated and complex host-entering behaviour, which leads the worm to enter inside the host bivalve mainly through the inhalant siphon (Martin et al., 2015). Moreover, the presence of *O. okupa* sp. nov. caused a significant reduction in the soft-body biomass of the infested hosts, compared to the non-infested ones, which may imply affectation of the host's metabolism according to Bierbaum and Ferson (1986) thus leading to a relationship closer to parasitism (Martin et al., 2012). Such a negative influence has been previously reported for other symbiotic polychaetes living in the mantle cavity of bivalves, such as the deep-sea hydrothermal vent polynoids *B. seepensis* (Britayev et al., 2007). However, contrary to *B. seepensis*, in the case of *O. okupa* sp. nov. no damages in the tissues of *S. plana* were observed (Martin et al., 2012). In *B. seepensis*, tissue damages were considered as a secondary effect of the worm's feeding inside the host caused by powerful jaws of the polynoid, and not a voluntary ingestion. *Oxydromus okupa* sp. nov. lacks jaws so that, even in the case of having a feeding mode similar to that *B. seepensis*, the hesionid seems to be able to avoid causing involuntary damage to the host tissues.

Despite the overall growing knowledge on symbiotic hesionids, *O. okupa* sp. nov. is probably the best known representative of the group to date. Nevertheless, further studies are required to complete the knowledge on this species, which becomes apparent if we compare it with *O. humesi*, with which it shares a similar morphology and a closely related host that is several thousands of kilometres away from Cadiz Bay. In other words, the present study is just another brick in the wall, which hopefully will encourage further research on the complex relationships between the symbiotic species of *Oxydromus* and their hosts.

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Appendix

In this section, the specimens of *Oxydromus* living symbiotically with the tellinid bivalves *Scrobicularia plana* and *Macoma cumana* in the Atlantic coasts of the Iberian Peninsula are described as a new species. The new species was previously confused with the congeneric *Oxydromus humesi*, which also lives symbiotically with a tellinid bivalve, *Tellina nymphalis*, in Congolese Atlantic coasts. To allow comparisons, a morphometric diagnosis of *O. humesi* and a general morphological and morphometric descriptions of the new species are provided. The type series of the two species are deposited at the Smithsonian Museum (USNM), the Museo nacional de Ciencias Naturales of Madrid (MNCN), the Annelida Polychaeta research collection of the Centre d'Estudis Avançats de Blanes (CEAB.A.P.), the Museu Nacional de História Natural e da Ciência of Lisboa (MUHNAC), the Biological Research Collection of the Departamento de Biologia of the Universidade de Aveiro (DBUA), the Senckenberg Forschungsinstitut und Naturmuseum of Frankfurt (SMF) and the National Museum of Wales, Cardiff (NMW).

Taxonomic account

Family Hesionidae Grube, 1850
Genus *Oxydromus* Grube, 1855

Oxydromus humesi (Pettibone, 1961)

LSID: urn:lsid:zoobank.org:act:
8453A918-89A8-4EEB-B78D-F9C452A39FA5

Parasyllidea humesi Pettibone, 1961. Pettibone (1961): figs. 4a–4h.

Oxydromus humesi (Pettibone, 1961). Martin et al. (2015) 434–441, figs. 4 (A, C, E), 5 (C, D, E, H) – 6 (A, C) – 7.

Material examined. Paratypes: USNM 30012, ca. 50 specimens fixed in formalin and preserved in 70% ethanol, Loango, Republic of Congo. (West Africa, approximately 4°37'42.6", 11°49'30.0"); intertidal, mangrove muddy bottoms.

Morphometric diagnosis. Based on the average proportions being significant different from *O. okupa* sp. nov. (Table 8); “long” and “short” refer to parapodia of chaetigers 10 and 15, bearing long and short cirrostyles,

respectively. General morphological description same as *O. okupa* sp. nov. Ratios number of segment and body width (without parapodia) vs. body width (with parapodia) ca. 0.03 and 0.5, respectively. Lateral antennae, palpophores and palpostyles ca. 0.5, 0.25 and 0.4 as long as prostomium length, respectively. Distance between anterior and posterior eyespots ca. 0.21 as long as prostomium length. Distance between anterior eyespots ca. 1.06 as long as that between posterior ones. Long and short dorsal cirrostyles 9 and 8 as long as cirrophores and 1.5 and 1.2 as long as dorsal lobes, respectively. Ventral lobe twice as long as dorsal one. Serration near blade tip less than twice short as blade width.

Oxydromus okupa Martin, Meca and Gil sp. nov.

LSID: urn:lsid:zoobank.org:pub:
D97B28C0-4BE9-4C1E-93F8-BD78F994A8D1

Figures 7–9

Parasyllidea humesi [non Pettibone, 1961]. Martin et al. (2012): 147–148, fig. 2b–2c;
Oxydromus humesi [non (Pettibone, 1961)]. Martin et al. (2015): 434–441, figs. 3, 4 (B, D, F), 5 (A, B, F, G), 6 (B, D), 7.

Material examined: Holotype MNCN 16.01/17760, 1 specimen. Paratypes: MNCN 16.01/17761, 24 specimens. Collected at Río San Pedro (Cádiz Bay, southern Atlantic coast of the Iberian Peninsula, 36°31'56.28", 6°12'53.28"), intertidal muddy bottoms. All specimens fixed in a 4% formalin–seawater solution and preserved in 70% ethanol.

Additional material: Paratypes: MNCN 16.01/16090, 20 specimens, and CEAB.A.P. 854 A, 20 specimens. MUHNAC, MB29-000343 to MB29-000357, 15 specimens; DBUA0002020.01 to DBUA0002020.13, 13 specimens; SMF 24348 to SMF 24353, 6 specimens; NMW.Z.2017.010.0001 to NMW.Z.2017.010.0006, 9 specimens. Collection, fixation and preservation as for holotype.

General morphological description: Body rusty orange to tan (pale yellowish when preserved), flattened dorso-ventrally, tapering gradually posteriorly. Prostomium subrectangular, without posterior incisions, with two

Fig. 7. *Oxydromus okupa* sp. nov. Light microscopy micrographs of the holotype in dorsal view. a. Whole worm. b. Anterior mid-body. c. Anterior end. Scale bars are μm .

Fig. 8. Light microscopy micrographs of paratypes in dorsal view. *Oxydromus okupa* sp. nov. a. Whole worm. b. Anterior mid-body. c. Anterior end. *Oxydromus humesi*. d. Whole worm. e. Anterior mid-body. f. Anterior end. Scale bars are μm .

pairs of small reddish-brown eyes (dark brown when preserved), a median ridge, biarticulated palps and three smooth antennae inserted frontally; palpophores cylindrical, palpostyles flask-shaped, about twice the length of palpophores; median antenna very small, button like; lateral antennae conical, with tapering tips. Nuchal organs on lateroposterior prostomial margin.

Facial tubercle present. Lip pads absent. Proboscis with two smooth rings and a terminal ciliated one. Jaws absent. Segment 1 dorsally reduced. Parapodial lobes and chaetae absent on segments 1-3. Long and short dorsal cirrophores distinct, ventral ones often indistinct. Dorsal and ventral cirrostyles smooth, distally tapering, slightly alternating short and long, ventral cirri shorter

Fig. 9. *Oxydromus okupa* sp. nov. a. Chaetiger 10, posterior view. b. Chaetiger 30, posterior view. c. Typical articulated chaeta. d. Tip of articulated chaeta. *Oxydromus humesi*. e. Chaetiger 10, posterior view. f. Chaetiger 30, posterior view. g. Tip of articulated chaeta. Scale bar is μm .

than dorsal, with blunt tip. Parapodia subbiramous from segment 4 onwards; notopodial lobe achaetous, half as long as neuropodial one, alternating elevated and non-elevated, with cirrophore on tip, with up to five aciculae; neuropodial lobes chaetigerous, elongated, subconical, with blunt tips, with long digitiform prechaetal lobe, rounded postchaetal lip, up to three aciculae and numerous compound neurochaetae, with heterogomph shafts, unidentate blades (middle ones much longer than dorsal and ventral) with curved, blunt tips, finely denticulate cutting edges, and a long subdistal spine reaching tip of curved end. One pair of pygidial cirri, similar to dorsal cirri. Pygidial papilla absent. Anus terminal.

Morphometric description. This description is based on the holotype (MNCN 16.01/17760); “long” and “short” refer to parapodia of chaetigers 10 and 15, bearing long and short cirrostyles, respectively). Tables 3 and 6 summarizing population ranges and averages. Body 11.6 mm long, 2 mm wide at the level of chaetiger 10, with 48 segments. Body width (without parapodia) ca. 0.35 as wide as with parapodia, ca. 5.8 as wide as body length. Ratio number of segments vs. body width (with parapodia) = 0.02. Prostomium 287 μm long and 499 μm wide; lateral antenna 226 μm long; palpostyles almost twice as long as palpophores, 150 μm and 74

μm long, respectively. Lateral antennae (226 μm long), palpophores (74 μm long) and palpostyles (159 μm long) 0.8, 0.3 and 0.5 as long a prostomium length, respectively. Distance between anterior eyespots 386 μm ; distance between posterior eyespots 356 μm ; distance between anterior and posterior eyespots 61 μm . Distance between anterior eyespots ca. 1.09 as long as that between posterior ones. Distance between anterior and posterior eyespots ca. 0.7 and 0.8 as long as prostomium width, respectively. Distance between anterior and posterior eyespots ca. 0.21 as long as prostomium length. Long and short dorsal lobes similar in size, ca. 210 μm long, tips almost reaching those of ventral lobes. Long and short dorsal cirrophores 41 and 30 μm long, 0.14 and 0.2 as long a dorsal lobe. Long and short dorsal cirrostyles 530 and 512 μm long, ca. 2.4, 2.5 as long as dorsal lobes, and 17.8 and 12.8 as long as cirrophores. Long and short posterior neurochaetal lobe similar in size, ca. 136 μm long. Long and short ventral cirri 206 and 198 μm long, ca. 1.5 as long as posterior neurochaetal lobes.

Remarks: *Oxydromus okupa* sp. nov. and the morphologically-similar *O. humesi* share a virtually identical general morphological description, which resulted in them being initially identified as the same species (Martin et al. 2015). There was a single chaetal type, compound ones with heterogomph unidentate blades, which were very similar in both species. The blade serration seemed to be longer in the new species than *O. humesi*, although this character is highly variable and depends on the observed chaetae so that we strongly suggest not relying on it to differentiate the two species. Specimens corrected for body size (as body width including parapodia) show significant quantitative differences in body measurements (Table 6). Among these differences, the new species has significantly fewer segments and a narrower body width (without parapodia). The head is almost 4% wider, and the cephalic appendages are significantly longer in *O. okupa* sp. nov., with the latter including dimensions of palpophores (12% longer), palpostyles (14% longer), lateral antennae (19% longer) and distances between eyes (particularly between the anterior and posterior pairs). Some parapodial measurements are also significantly longer (6 to 9.6% long), while the dorsal lobe is the only character being shorter in *O. okupa* sp. nov. (8.5% and 13% shorter in parapodia bearing long and short cirrostyles, respectively). Moreover, dorsal and ventral lobes are similar in length, with the tip of the former reaching the tip of the latter in *O. okupa* sp. nov., while the dorsal

lobe is much shorter than the ventral one in *O. humesi*.

Oxydromus okupa sp. nov. differs from *O. humesi* in body measurement proportions (Table 8). Accordingly, *O. okupa* sp. nov. has the body width measured without parapodia proportionally narrower with respect to the body width measured with parapodia, and it has also fewer segments. The new species also has the cephalic appendages proportionally longer with respect to prostomium length, particularly in the case of lateral antennae (more than 16% longer), and the distance between anterior and posterior eye pairs is also significantly longer, too. All ratios concerning parapodial measurements are also higher for *O. okupa* sp. nov., with the most marked differences being at the level of the dorsal cirrostyle vs. the corresponding dorsal lobe (15-22%) and the cirrophore (9-13.5%). Serration near blade tip as long as blade width.

Etymology: The specific epithet “*okupa*” refers to the *Okupas* (i.e., the Spanish squatters), a movement that emerged in the 80s as a response to the ever-increasing difficulty of housing opportunities. *Okupation* means squatting in empty buildings for the own use of the *okupas*, which implies self-organisation and alternative relationship mechanisms, in a parallel way as symbiotic polychaetes interact with their hosts and with the whole ecosystem.

Geographical distribution: Despite the wide distribution of *S. plana* in European waters (Santos *et al.*, 2011), *Oxydromus okupa* sp. nov. is currently only known from the type locality, the intertidal of Río San Pedro saltmarshes, and the nearby subtidal environments of Cádiz Bay (southern Atlantic coast of the Iberian Peninsula).