Disentangling the determinants of symbiotic species richness in native and invasive gammarids (Crustacea, Amphipoda) of the Baltic region

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ABSTRACT

Dispersal of alien species is a global problem threatening native biodiversity. Co-introduction of non-native parasites and pathogens adds to the severity of this threat, but this indirect impact has received less attention. To shed light on the key factors determining the richness of microorganisms in native and invasive host species, we compared symbiotic (parasitic and epibiotic) communities of gammarids across different habitats and localities along the Baltic coast of Poland. Seven gammarid species, two native and five invasive, were sampled from 16 freshwater and brackish localities. Sixty symbiotic species of microorganisms of nine phyla were identified. This taxonomically diverse species assemblage of symbionts allowed us to assess the effect of host translocation and regional ecological determinants driving assembly richness in the gammarid hosts. Our results revealed that (i) the current assemblages of symbionts of gammarid hosts in the Baltic region are formed by native and co-introduced species; (ii) species richness of the symbiotic community was higher in the native *Gammarus pulex* than in the invasive hosts, probably reflecting a process of species loss by invasive gammarids in the new area and the distinct habitat conditions occupied by *G. pulex* and invasive hosts; (iii) both host species and locality were key drivers shaping assembly composition of symbionts, whereas habitat condition (freshwater versus brackish) was a stronger determinant of communities than geographic distance; (iv) the dispersion patterns of the individual species richness of symbiotic communities were best described by Poisson distributions; in the case of an invasive host, the dispersion of the rich species diversity may switch to a right-skewed negative binomial distribution, suggesting a host-mediated regulation process. We believe this is the first analysis of the symbiotic species richness in native and invasive gammarid hosts in European waters based on original field data and a broad range of taxonomic groups including Microsporidia, Choanozoa, Ciliophora, Apicomplexa, Platyhelminthes, Nematoda, Nematomorha, Acanthocephala and Rotifera, to document the patterns of species composition and distribution.

1. Introduction

Economic globalisation has facilitated the interconnection between the world's territories, accelerating species migration rates and the spread of disease-causing organisms (Saebi et al., 2020; Jeanne et al., 2022). The annual rate of first records of alien species worldwide has increased steadily during the last 200 years, with 37% of all first records reported in the last four decades (Seebens et al., 2017). The explosive growth of invader populations in the colonized areas poses a potential threat to native species, ecosystem processes, community structure, human and wildlife health, and may cause substantial economic losses (Simberloff et al., 2013). Although not all alien species may have such direct negative impacts on their recipient systems, the translocation of non-native parasites and pathogens with their host represents an additional threat, since successful invaders may carry with them multiple symbiotic organisms (Vilcinskas, 2015; Young et al., 2017; Bojko et al., 2021). If introduced symbiotic agents surmount survival and reproductive barriers, they establish a new population and may spill over or not to native hosts to become co-invasive or co-introduced, respectively (Lymbery et al., 2014). The first

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encounters of a native host with an introduced pathogen, or of a native pathogen with an introduced host, typically lead to pathogenicity in the new host system due to the absence of a common evolutionary history. Co-invasive pathogens and parasites have been proposed to act as biological weapons of invasive species if they infect and kill native competitors (Vilcinskas, 2015). For instance, the invasive Asian nematode *Anguillicola crassus* was responsible for the massive decline in recruitment of European eel (*Anguilla anguilla*) (Sures and Knopf, 2004); the introduction of the North American crayfish has been related to the spread of crayfish plague (*Aphanomyces astaci*) to native European crayfish species, resulting in steep population declines (James et al., 2017). However, this indirect impact of pathogen co-invasions has received less attention and therefore analyses of the invasion process from this perspective are much needed (Sarabeev et al., 2022a).

Retrospective studies on the formation of symbiotic communities in introduced macroorganisms show that species composition can originate from three possible locations: (i) the invasive species' native range, (ii) an intermediate location between the invasive species' native range and its current distribution area, and (iii) the region where the invasive species becomes established (Chalkowski et al., 2018; Llopis-Belenguer et al., 2020a; Sarabeev et al., 2022a). Despite the variety of sources accounting for symbiotic communities of introduced macroorganisms, the success of alien species is most frequently explained by the enemy release hypothesis (ERH). This hypothesis is based on predictions that alien species leave behind competitors and/or enemies in their native range (Torchin and Lafferty, 2009; Chalkowski et al., 2018). Modern understanding of the processes governing species assemblies in communities assumes that species distributions would be mostly constrained by local environmental conditions, historical large-scale events, dispersal capacity of the species and interspecies interaction (Pavoine and Bonsall, 2011; Liautaud et al., 2019; Llopis-Belenguer et al., 2020b). Although symbionts are fed and sheltered by the host organism, co-introduced species should also overcome these ecological filters to become established in the new distribution range (Wattier et al., 2007). Usually, most if not all co-translocated symbionts of alien species do not survive in the new environment, while native naïve parasites also may fail to infect a new host due to closed compatibility filters, altogether leading to the enemy release (Combes, 2001; Torchin and Lafferty, 2009; Lymbery et al., 2014; Chalkowski et al., 2018; Sarabeev et al., 2019; Llopis-Belenguer et al., 2020b). Thus, uncovering the determinants shaping the species composition of symbiotic microorganisms in non-native hosts is needed for a firm understanding of the invasion process and factors influencing the symbiotic community composition of alien hosts (Wattier et al., 2007).

Three distinct, but not mutually exclusive, hypotheses can be formulated based on the evidence from parasitic and free-living organisms:

(1) Because virtually all metazoan species harbour a number of symbionts, parasites and pathogens can be translocated with them into a new area (Dunn, 2009; Lymbery et al., 2014; Bojko et al., 2021). Thus, we hypothesize that the richness of symbiotic organisms in the new area will increase.

(2) According to the ERH (Torchin and Lafferty, 2009; Chalkowski et al., 2018), species richness of symbiotic communities of the invasive hosts should be lower than that of the native hosts.

(3) Both host phylogeny and geographic localities will influence the richness of parasite communities, while habitat conditions will be a stronger determinant of the parasite communities than geographic distance (Llopis-Belenguer et al., 2020b; Moss et al., 2020; McNew et al., 2021).

The present study focuses on parasitic and epibiotic symbionts of invasive and native gammarid species in fresh and brackish waters of the Baltic region of Poland. In total, 21 species of gammarids are known to occur along the Polish coast of the Baltic Sea. At least nine of them are alien species. Seven are Ponto-Caspian: *Echinogammarus ischnus* (Stebbing), *Dikerogammarus haemobaphes* (Eichwald), *Dikerogammarus villosus* (Sovinsky), *Pontogammarus robustoides* (G.O. Sars), *Obesogammarus crassus* (G.O. Sars), *Gammarus varsoviensis* Jazdzewski, *Echinogammarus trichiatus* (Martynov); one is North American, *Gammarus tigrinus* Sexton, and one is of Balkanic origin, *Gammarus roeselii* (Gervais). These species coexist with five native freshwater species *Gammarus pulex* (L.), *Gammarus lacustris* G.O. Sars, *Gammarus fossarum* Koch, *Gammarus leopoliensis* Jazdzewski and Konopacka and *Gammarus balcanicus* Schäferna; and seven native brackish species *Gammarus zaddachi* , *Gammarus locusta* (L.), *Gammarus duebeni* Lilljeborg, *Gammarus salinus* Spooner, *Gammarus inaequicauda* Stock, *Gammarus oceanicus* Segerstråle and *Echinogammarus stoerensis* (Reid) (Jazdzewski et al., 2002; Grabowski et al., 2012; Rachalewski et al., 2013; Dobrzycka-Krahel et al., 2019).

The present paper, with a special focus on species richness, initiates an investigation of symbiotic communities and populations from native and invasive gammarid hosts in the Baltic region of Poland using a recently proposed macroecological framework to study host-parasite relationships and a better understanding of the invasion process (Sarabeev et al., 2022a). Species richness is the simplest measure of diversity, it represents a measure of the variety of species based simply on a count of the number of species in a particular sample, although it can be expressed more usefully as species richness per unit area, ranging from α (at the host individual level) to γ (for an entire study area) level (Chakraborty et al., 2022). Despite increasing interest in parasites and pathogens of invasive amphipods in European waters, much of the research until now has been descriptive in nature (Ovcharenko et al., 2009, 2010, 2012; Bojko et al., 2017, 2018; Urrutia et al., 2019; Allain et al., 2020) or limited to one taxonomic group (Wattier et al., 2007; van der Velde et al., 2009; Bacela-Spychalska et al., 2012, 2014, 2018; Grabowski et al., 2012, 2017; Grabner et al., 2015; Bojko et al., 2018; Rachalewski et al., 2018; Quiles et al., 2019, 2020; Kobak et al., 2021). Our works preceding the present analytic study were also descriptive and represented a taxonomic overview of the revealed symbiotic species (Sarabeev et al., 2022b; Sarabeev and Ovcharenko, 2022). Here we compare symbiotic communities of gammarids, including a broad range of epibiotic and endobiotic organisms, across different habitats and localities along the Baltic coast of Poland to shed light on the key factors determining the richness of microorganisms in native and invasive host species.

2. Materials and methods

2.1. Material collection and processing

Gammarids were collected from 16 sampling localities in the Baltic region of Poland (Fig. 1; Table 1) to cover a range of rivers, streams, deltas and canals with a typical habitat of native and invasive amphipod species. Some of these water bodies were in a near pristine state (e.g. streams and upper flows of rivers), while others were subjected to varying degrees of anthropogenic impacts (e.g. eutrophication, canalization, and ship traffic). Hand net samples were taken in the cold season from October 2020 to April 2021. Fine sediments and small stones were sieved. If larger stones or wood substrates were present, they were turned to remove gammarids. The specimens were transported to the laboratory alive in plastic containers filled with aerated water and maintained in aerated aquaria under low temperatures (5–7 °C) before examina-
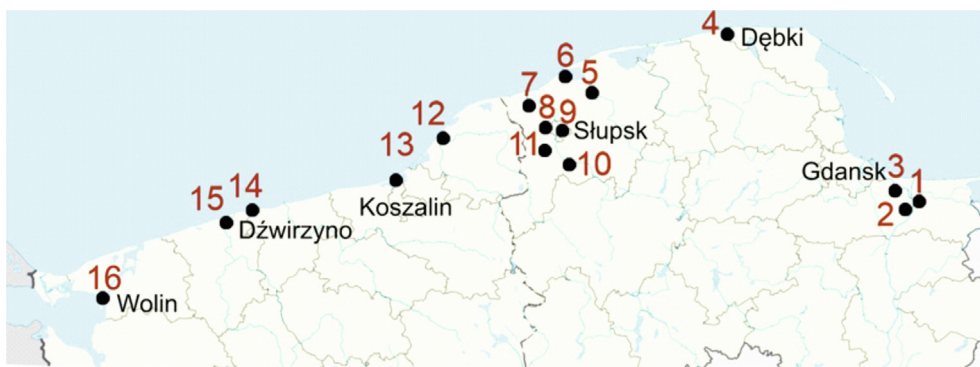
Fig. 1. Localities surveyed (with geographic coordinates in parenthesis) for symbionts of gammarids in the Baltic region of Poland: (1) WS, Wisla Sobieszewska (54°18′50.83″N, 18°55′54.49″E); (2) PL, Port Lodolamaczy (54°18′32.281″N, 18°55′30.774″E); (3) MW, Martwa Wisla (54°18′38.6″N, 18°52′2.01″E); (4) DP, Dębki Piasnicy (54°49′56.24″N, 18°3′42.67″E); (5) SL, Smoldzino Lupawa (54°39′43.5060″N, 17°12′44.1556″E); (6) RC, Rowy Canal (54°40′2.24″N, 17°3′24.93″E); (7) O, Orzechowa (54°35′55.8″N, 16°55′7.8″E); (8) SW, Wodnica Slupia (54°33′23.84″N, 16°52′30.83″E); (9) SS, Słupsk Stream (54°28′30.936″N, 17°2′34.222″E); (10) LD, Lesny Dwor (54°21′30.606″N, 17°9′20.567″E); (11) KS, Krępa Słupska (54°24′12.32″N, 17°2′49.24″E); (12) DW, Darlowko Wieprza (54°25′60.598″N, 16°23′20.622″E); (13) JN, Jamiensky Nurt (54°16′54.18″N, 16°8′8.09″E); (14) DR, Dźwirzyno Regoujście (54°9′10.9786″N, 15°23′25.9124″E); (15) MP, Mrzeżyno Pera (54°8′27.5568″N, 15°17′4.8048″E); (16) WD, Wolin Dziwna (53°50′25.1048″N, 14°37′18.6744″E).

Table 1
Date, locality, habitat characteristic, number of hosts (n) and total ($\beta1$) and individual (α) species richness of symbiotic microorganisms of gammarids sampled from fresh (FW) and brackish (BW) water bodies in the Baltic region of Poland.

Host species	Date	Locality	Habitat	Sample code	n	$\beta1$ community richness			α community richness		
						Endobiotic	Epibiotic	Whole	Endobiotic Mean ± S. D.	Epibiotic Mean ± S. D.	Whole Mean ± S. D.
Dikerogammarus villosus	28.03.2021	Jamiensky Nurt	FW, canal	DVJN	30	5	10	15	1.36 ± 0.49	3.96 ± 1.48	5.07 ± 1.66
D. villosus	05.04.2021	Mrzeżyno Pera	FW, delta	DVMP	30	6	10	16	1.82 ± 0.73	4.00 ± 1.05	5.33 ± 1.37
D. villosus	18.11.2020	Port Lodolamaczy	FW, port	DVPL	30	6	10	16	1.96 ± 0.86	2.39 ± 1.37	3.93 ± 1.58
D. villosus	10.01.2021	Rowy Canal	BW, canal	DVRC	30	6	11	17	1.50 ± 0.80	3.73 ± 1.28	4.33 ± 1.67
D. villosus	18.11.2020	Wisla Sobieszewska	FW, delta	DVWS	30	7	11	18	1.58 ± 0.69	3.37 ± 1.30	4.37 ± 1.63
D. villosus	All samples				150	12	21	33	1.67 ± 0.74	3.50 ± 1.41	4.60 ± 1.65
Echinogammarus ischnus	15.03.2021	Wolin Dziwna	FW, delta	EIWD	25	2	7	9	1.00 ± 0.00	3.08 ± 1.28	3.25 ± 1.48
Pontogammarus robustoides	05.04.2021	Wisla Sobieszewska	FW, delta	PRWS	38	7	13	20	1.67 ± 0.76	2.42 ± 1.23	3.53 ± 1.56
Gammarus roeselii	06.03.2021	Darlowko Wieprza	FW, delta	GRDW	30	7	13	20	1.68 ± 0.90	4.66 ± 2.92	6.07 ± 2.96
Gammarus tigrinus	05.04.2021	Dźwirzyno Regoujście	BW, canal	GTDR	30	6	6	12	1.65 ± 0.49	2.66 ± 1.04	4.00 ± 1.44
G. tigrinus	18.11.2020	Martwa Wisla	BW, delta	GTMW	30	3	2	5	1.05 ± 0.22	1.22 ± 0.42	1.93 ± 0.72
G. tigrinus	31.01.2021	Rowy Canal	BW, canal	GTRC	26	5	9	14	1.02 ± 0.50	2.41 ± 1.05	2.74 ± 1.30
G. tigrinus	All samples				86	9	11	20	1.35 ± 0.48	2.12 ± 1.04	2.95 ± 1.44
Gammarus pulex	20.02.2021	Debki Piasnicy	FW, river	GPDP	30	5	11	16	1.63 ± 0.65	5.03 ± 1.27	6.33 ± 1.56
G. pulex	27.04.2021	Krępa Słupska	FW, stream	GPKS	41	11	9	20	2.85 ± 1.33	3.95 ± 1.24	6.80 ± 2.08
G. pulex	10.10.2020	Lesny Dwor	FW, river	GPLD	28	7	9	16	1.95 ± 1.00	2.92 ± 1.32	4.11 ± 1.89
G. pulex	21.10.2020	Orzechowa	FW, stream	GPO	34	9	16	25	1.63 ± 0.68	4.43 ± 2.47	5.17 ± 2.68
G. pulex	31.01.2021	Smoldzino Lupawa	FW, river	GPSL	31	9	15	24	1.93 ± 0.98	7.63 ± 1.90	9.43 ± 2.11
G. pulex	28.02.2021	Slupsk Stream	FW, stream	GPSS	30	10	13	23	2.00 ± 0.85	4.00 ± 1.02	5.73 ± 1.11
G. pulex	25.03.2021	Wodnica Slupia	FW, river	GPSW	30	7	11	18	1.59 ± 0.68	4.03 ± 1.09	5.62 ± 1.24
G. pulex	All samples				224	18	27	45	2.02 ± 1.05	4.53 ± 2.04	6.19 ± 2.40
Gammarus zaddachi	31.01.2021	Rowy Canal	BW, canal	GZRC	30	6	11	17	1.10 ± 0.32	2.97 ± 1.45	3.34 ± 1.56
G. zaddachi	05.04.2021	Dźwirzyno Regoujście	BW, canal	GZDR	29	10	11	21	1.79 ± 0.66	2.41 ± 1.38	3.90 ± 1.45
G. zaddachi	All samples				59	13	15	28	1.59 ± 0.66	2.69 ± 1.43	3.62 ± 1.52

tion for symbionts. The parasite surveys were done within several working days after field collection.

The gammarids were morphologically identified, measured and dissected under a stereomicroscope on an object glass slide. The number of specimens dissected typically reached 30 specimens per sample according to the recommendations of Marques and Cabral (2007), and Shvydka et al. (2018). Each anatomical unit (body surface with muscles, intestine, body cavity organs, gills) was prepared separately and observed under a compound microscope. All symbionts detected were counted, registered and microphotographed. Taxonomic identification was attempted to the lowest possible level. The present study explores metazoan parasites (Platyhelminthes, Nematoda, Nematomorha, Acanthocephala and Rotifera), protozoans (Ciliophora and Apicomplexa (Eugregarina)), fungi and fungi-like organisms (Microsporidia and Choanozoa, respectively).

Microsporidians and digenean metacercariae were identified both morphologically and via a partial sequence of the ssrRNA gene, while the remaining symbiotic groups were identified only morphologically. Ciliophorans were identified with keys proposed by Curds (1985), Warren (1986, 1988) and Dovgal (1996), and published species descriptions (De Puytorac and Lom, 1962; Fahrni, 1983; Schödel, 1983, 2018; Clamp, 1991; Dovgal and Mayén-Estrada, 2013; Mayén-Estrada and Clamp, 2016; Lynn and Strüder-Kypke, 2019). Other taxa were identified as follows: digeneans according to Deblock (2008), Tkach (2008), and Sarabeev et al. (2022b); acanthocephalans according to Lisitsyna (2019); cestodes according to Bojko and Ovcharenko (2019); nematodes followed Moravec (1998); rotifers according to Jersabek (2002), and Smet and Verolet (2016); nematomorpha followed Bolek et al. (2015); choanozoans according to Moss (1999); apicomplexans according to Poisson (1921, 1924), Bălcescu (1972), Bălcescu-Codreanu (1974), Codreanu-Bălcescu (1995, 1996); microsporidians according to Larsson (1983), Friedrich et al. (1995), Terry et al. (1999), Issi et al. (2010), Ovcharenko et al. (2010), and Bacela-Spychalska et al. (2018). The species list of identified symbionts is shown in Supplementary Table S1 with information on symbiotic relationships, infection sites and host specificity.

2.2. Data analysis

Unless otherwise stated, all analyses were carried out in the R environment (https://www.r-project.org). The analysis of richness was carried out at the two hierarchical levels, the infra-community (α richness) and the component community (β richness). The former represents the species richness of the symbiotic community at the individual host level, while the latter is species richness at the level of host populations or communities (Holmes and Price, 1986; Llopis-Belenguer et al., 2020b). So β richness can be measured at two levels, host population ($\beta1$ richness) and host community or host species ($\beta2$ richness). The mean individual α richness ± S.D. and the total $\beta1$ richness were computed for each sample and are presented in Table 1. The raw α richness for each studied host individual is presented in Supplementary Table S2. The analyses were applied to three subsets of data: the whole symbiotic, the ectosymbiotic and the endosymbiotic communities. The last two groups differ not only in the infection site but in the forms of relationships with their hosts. Ectosymbiotic species, also called epibionts, are in neutralistic or commensalistic relationships with hosts, while endosymbiotic organisms are largely parasites. The exception is intestinal gregarines whose status may vary between the boundaries of mutualism and parasitism as their pathogenicity and influence on the host population's ecology are still in question (Rueckert et al., 2019).

2.2.1. β richness

Since host sample size differed among species, the $\beta2$ richness of symbiotic communities in host species was compared using rarefaction curves (Gotelli and Colwell, 2001, 2011). Rarefaction represents an interpolation of a biodiversity sample to a smaller number of individuals for comparison among samples. Samples from different hosts were rarefied to the smallest sample represented by 25 individuals of *E. ischnus* to determine if the total species richness of microorganisms differs for a common number of host individuals. Sample-based rarefaction curves (using each crustacean individual as a sample) and corresponding 95% confidence intervals were obtained with EstiMateS (v. 9.1.0) (http://purl.oclc.org/estimates).

To evaluate the relationships between $\beta1$ species richness and host species, locality and habitat, simple linear regression models with subsequent F-tests were fitted over 20 samples defined by host species and localities. Linear regressions were applied under the assumptions of independence and normality of the dependent variables.

The similarity in the total symbiotic species richness was assessed using the Whittaker's index (Whittaker, 1960) as applied in the "vegan" package (https://cran.r-project.org/web/packages/vegan/index.html). The matrix of similarity obtained was used to cluster samples based on the data of $\beta1$ richness of symbiont communities. Dendrograms were constructed using the unweighted pair-group mean average method. Whittaker's index matrix based on species occurrence in between 20 samples was obtained with PAST (v. 4.12) (Hammer et al., 2001) and is presented in Supplementary Table S3.

2.2.2. Alpha richness

Since the dispersion pattern of individual species richness of symbionts can serve as a first-line indicator of the antagonistic host-parasite relationships of the invasive host with acquired symbionts (Sarabeev et al., 2022a), the dispersion was estimated using two methods. First, the variance to mean ratio was computed. This parameter varies from zero (when all hosts harbour exactly the same number of species), through one (the number of species in the hosts is random), to a number equal to the total number of species (for a maximally aggregated distribution of the number of species) (Wilson et al., 2002). Second, we tested the fit of α richness to the Poisson model, which is the most expected dispersion pattern of individual species richness (Poulin, 1997, 1996), and alternatively to the negative binomial and normal distributions. The "goodfit" and "shapiro.test" functions of the 'goft' package v. 1.3.6 (https://cran.r-project.org/web/packages/goft/index.html) were used.

In order to examine the relationship between α richness and host species, locality or habitat, we used the "glm" function with the Poisson family of distribution ("GLMpack" v. 0.1.0; https://cran.r-project.org/web/packages/GLMpack/index.html) that performs a one-way generalized linear model (GLM) for outcomes following Poisson distribution and provides empirical sampling distributions for further ANOVAs. Then, we performed ANOVAs with χ^2 tests. When differences between samples from different host species or localities were significant, we ran pairwise comparisons of α richness between host species or localities using the "aov" function and multiple comparison plot "mc_plot" in the PairViz package v. 1.3.5 (https://cran.r-project.org/web/packages/PairViz/index.html). In the case of co-habiting *G. zaddachi* and *G. tigrinus* in the Dźwirzyno Regoujście and Rowy Canal, two-way GLM with the Poisson family of distribution was performed to evaluate the effect of locality, host species and possible their interaction on α richness of microorganisms.

2.3. Data accessibility

Associated raw data are available via Mendeley Data, https://doi.org/10.17632/c9fzyyzr52.1 and were explained more precisely in Sarabeev et al. (2023). Briefly, three Microsoft® Excel files were stored: file #1 represents the raw data on the number of individuals (infrapopulation size) of each eukaryotic symbiont taxa recorded in each host individual and location; file #2 reports the symbiotic species list with information on host species, sampling date, locality and geographic coordinates, infection site, obtained sequences (if the case), brief morphological characteristics and microphotographs; file #3 provides measured water parameters, habitat features and host density per sample. Six tables with interim results of the richness analysis are included in Supplementary Tables S1-S6, and graphical data in Supplementary Figs. S1-S8, all included on-line with the present paper.

3. Results

3.1. Structure of symbiotic communities of microorganisms

In total 612 individuals of seven gammarid species were sampled from 16 localities in the Baltic region of Poland, representing 20 host-locality samples (Fig. 1; Table 1). Sixty symbiotic species of nine phyla were identified in all samples (Supplementary Table S1; Mendeley data, https://doi.org/10.17632/c9fzyyzr52.1, File 2). Almost half (29) of the symbiotic species corresponded to the Ciliophora. The remainder belonged to the Apicomplexa (12 species, 20%), Microsporidia (8, 13%), Platyhelminthes (3, 5%), Acanthocephala, Nematoda and Rotifera (2, 3%, each), and Choanozoa and Nematomorpha (1, 2%, each). Seven ciliate species (*Paracollinia branchiarum*, *Epistylis kolbi*, *Epistylis gammari*, *Zoothamnium hyalinum*, *Zoothamnium gammari*, *Intranstylum* sp. 2 and *Pseudocarchesium steinii*) accounted for 98.7% of the symbiotic specimens collected. Of these, the most abundant by far (47% of all symbiotic individuals) was *P. branchiarum*, a specific parasitoid infecting the hemocoel of *G. pulex*. Thirty-seven species (62%), all the ciliates except for two species, all helminth larvae and rotifers, can be regarded as non-specific. The remaining 23 species (38%) of Api-

complexa, Microsporidia, Choanozoa and the ciliates *P. branchiarum* and *Dendrocometes paradoxus* are stenoxenic or oioxenic symbionts related to gammarid hosts. The total species richness of epibiotic and endobiotic symbionts was 30 in both cases.

All gammarid species were infected with three colonial sessilid ciliates *Pseudocarchesium steinii*, *Epistylis kolbi* and *Zoothamnium parasiticum*. The first ciliate species was reported in all samples and localities except for Martwa Wisla. Among the endobiotic species, *Cephaloidophora gammari* and *Uradiophora longissimi* infected all host species except for *G.tigrinus* and *E. ischnus*. *Cephaloidophora gammari* was also reported in all localities except for Martwa Wisla. There was a relatively large number of symbiotic species that occurred only in one host species (21, 35%), locality (14, 23%) or in one sample (13, 22%). The 20 component communities defined by host species and locality varied greatly in taxonomic composition, although epibiotic ciliates and endoparasitic eugregarinorid gregarines occurred and dominated species richness in all samples (Fig. 2; Table 1). While choanozoans were represented by only one species, those were found in 12 samples. Microsporidians and rotifers were reported in 11 samples each.

3.2. Testing host, locality and habitat effects on β richness

The highest β2 richness of symbiotic community occurred in *G. pulex* (45 species), followed by *D. villosus* (33), *G. zaddachi* (28), *P. robustoides*, *G. roeselii* and *G. tigrinus* (by 20) and *E. ischnus* (nine) (Table 1). Although the γ (total) richness of endobiotic and epibiotic communities was equal, at the β2 level, the richness of endobiotic symbionts was much lower and accounted for 18 species in *G. pulex*, 13 in *G. zaddachi*, 12 in *D. villosus*, nine in *G. tigrinus*, seven in *P. robustoides*, seven in *G. roeselii* and two in *E. ischnus* versus 27, 15, 21, 11, 13, 13 and seven, respectively (Table 1). The richness of symbiotic microorganisms compared using the rarefaction curves standardized to the lowest sample in 25 host individuals showed a similar tendency for all subsets of data (Fig. 3). The richest whole symbiotic community was in *G. pulex*, followed by *D. villosus*, *G. zaddachi*, *G. roeselii*, *P. robustoides*, *G. tigrinus* and *E. ischnus*, although their 95% confidence intervals overlap extensively. The most species-rich locality was Orzechowa with 25 species, fol-
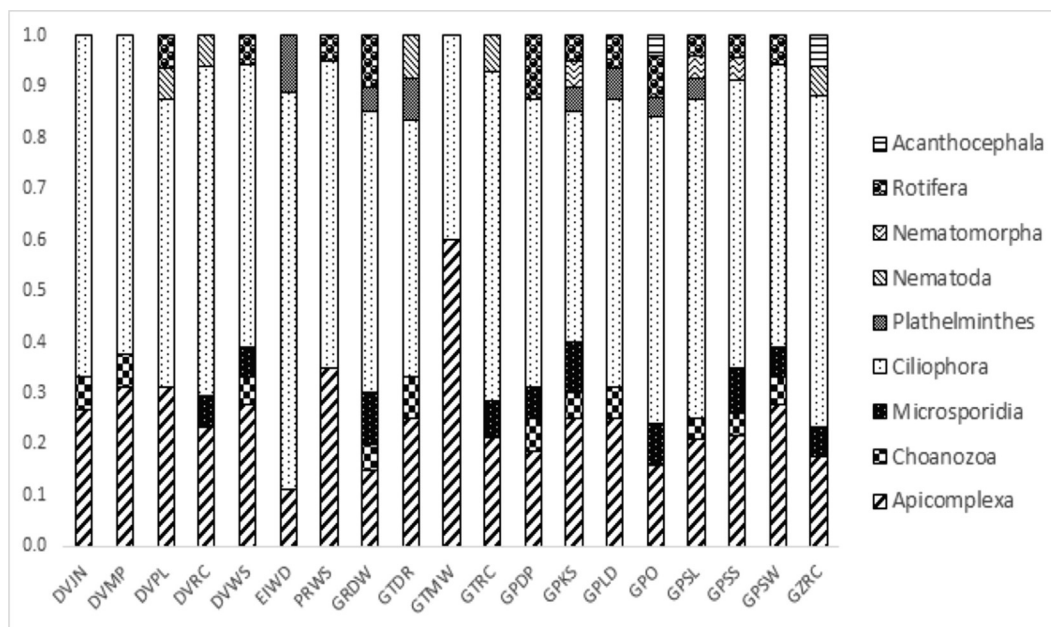

Fig. 2. Proportion of parasite species (relative β1 richness) grouped by phyla in 20 samples of gammarids defined by host species and sampling location in the Baltic region. Sample abbreviations as defined in Table 1.
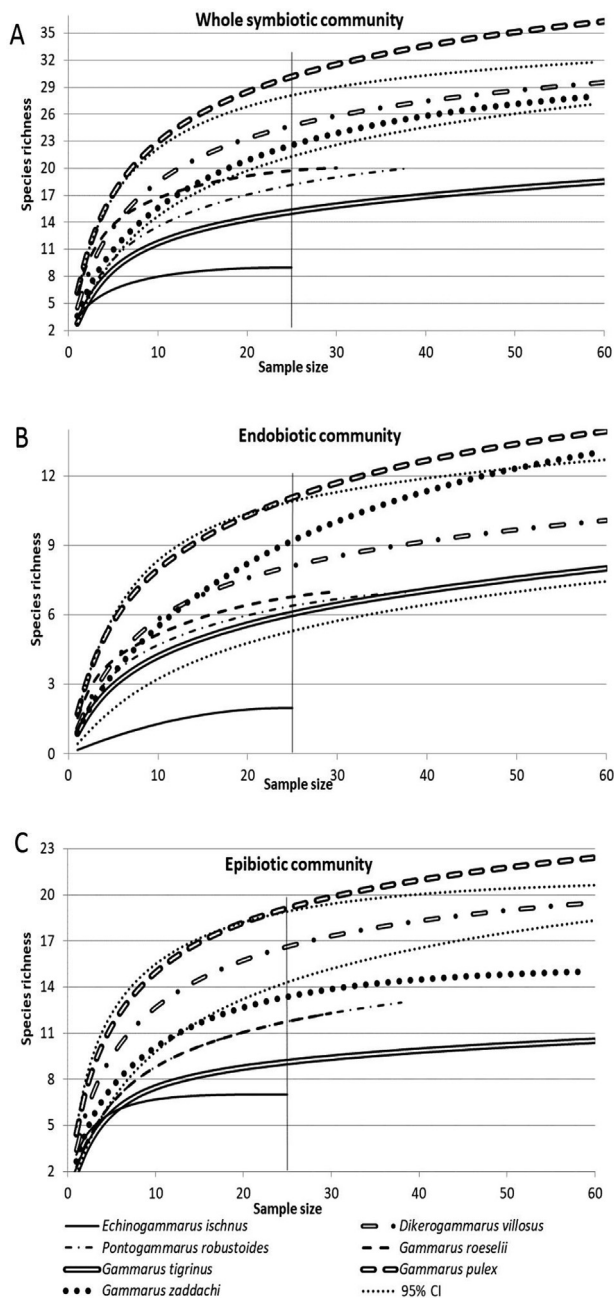
Fig. 3. Sample-based rarefaction curves for species β2 richness of whole symbiotic (A), endobiotic (B) and epibiotic (C) communities in each species of gammarid hosts from the Baltic region of Poland. Lower and upper bound 95% confidence intervals (CI) for the population of *Dikerogammarus villosus* are shown. Confidence intervals for the other hosts are not represented for the sake of clarity, but they overlap extensively. The vertical lines illustrate a species richness comparison standardized to 25 host individuals as the lowest sample for *Echinogammarus ischnus*.

lowed by Smoldzino Lupawa with 24 species, Słupsk Stream, Rowy Canal and Dźwirzyno Regoujście with 23 species in each. The lowest richness of symbiotic microorganisms was found in Martwa Wisla with only five species. Richness was higher in freshwater habitats than in brackish ones (the mean value of β1 counted over samples was 18 versus 14 species for the whole community, seven versus six species for the endobiotic community and 11 versus eight species for the epibiotic community). The linear regression analyses revealed a significant effect of host species on the richness of the whole, endobiotic and epibiotic communities (Table 2). The habitat effect was also significant in shaping the assemblages of

the epibiotic community, while the effect of localities was non-significant for any subset of data.

3.3. Clustering and similarity analysis of β1 richness

Symbiotic communities of microorganisms were most similar between *D. villosus* and *P. robustoides* from Wisla Sobieszewska (samples DVWS and PRWS, Whittaker's index 0.21, Supplementary Table S3), and least similar between *G. tigrinus* from Martwa Wisla and the other samples (0.75–1) (Fig. 4). There was moderate to the strong similarity between symbiotic communities among populations of *G. pulex* from different localities (0.26–0.56), *D. villosus* (0.29–0.56) and *G. zaddachi* (0.47), indicating that there was relative homogeneity of the composition of microorganism communities between host species within localities and among host populations of the same species from different localities but heterogeneity between host species sampled from different localities. Cluster analysis of the whole symbiotic communities of microorganisms (Fig. 4A), based on the Whittaker's index, grouped samples by localities (clades 1 and 3) and host species (clades 2 and 4). The same trend was observed for the epibiotic subset of data (Fig. 4B) and endobiotic communities (Fig. 4C). In the latter, this trend is less marked as samples were grouped by habitat (freshwater versus brackish, clades 1 and 2, Fig. 4C, respectively).

3.4. Distribution pattern of α richness

Multiple infections were common for gammarid hosts and reached the maximum of 14 species per host individual in *G. pulex* and *G. roeselii* from Smoldzino Lupawa and Darlowko Wieprza, respectively (Supplementary Figs. S1 and S2; counted raw data for α richness provided in Supplementary Table S2). The highest mean value and mode in seven symbiont species were also recorded for these two gammarid hosts. Among the tested theoretical distributions, the Poisson model best fitted the empirical dispersion of α richness in most cases, although a good fit to the negative binomial and normal distributions was found in some data sets (Table 3). The variance to mean ratio for the α richness was close to one in all but one case, which indicates a random distribution pattern of species richness across hosts. In epibiotic communities from *G. roeselii*, the variance to mean ratio was close to two, indicating an overdispersed distribution. The corresponding density plot of α richness showed a slightly right-skewed distribution with a relatively long tail (Supplementary Fig. S1).

3.5. Testing host, locality and habitat effects on α richness

The α and β species richness largely agreed with each other in the number of symbiotic species in the gammarid hosts, showing the highest richness in the native *G. pulex* and the lowest in the invasive hosts. The mean values of α richness were the highest in *G. pulex* (6.2 species per host individual), followed by *G. roeselii* (6.1), *D. villosus* (4.5), *G. zaddachi* (3.6), *P. robustoides* (3.3), *E. ischnus* (3.2), *G. tigrinus* (2.8) (Supplementary Fig. S1). The highest α richness was recorded in Smoldzino Lupawa in *G. pulex* (9.4 species per host individual) and the poorest in Martwa Wisla in *G. tigrinus* (1.9) (Table 1; Supplementary Fig. S2). The freshwater habitat is characterised by a richer community of symbiotics compared with the brackish water environment for the whole (mean values 5.3 versus 3.3), endobiotic (1.4 versus 0.8) and epibiotic (3.9 versus 2.5) communities. The one-way GLM performed over host individuals revealed a significant effect of all factors (host species, locality and habitat) on the α richness of all subsets of data (Table 2). Pairwise analyses between gammarid species found that α species richness of symbiotic communities from *G. pulex* and *G. roeselii* was significantly higher than from other hosts (Supplementary

Table 2
One-way ANOVA testing the effect of host, locality and habitat on β1 and α richness. The linear and generalized linear models were used for the analysis of β1 and α richness, respectively (see Supplementary Data S1 for code used in R).

Factor	Subset of data	β1 ($n = 20$)		α ($n = 612$)	
		F	P	Deviance	P
Habitat	Whole community	3.1	0.094	**111.8**	**2.2E-16**
	Endobiotic community	0.8	0.382	**38.5**	**5.5E-10**
	Epibiotic community	**4.5**	**0.048**	**74.1**	**2.2E-16**
Host	Whole community	**4.5**	**0.011**	**231.9**	**2.2E-16**
	Endobiotic community	**3.1**	**0.040**	**99.0**	**2.2E-16**
	Epibiotic community	**3.3**	**0.032**	**166.2**	**2.2E-16**
Locality	Whole community	1.9	0.281	**329.2**	**2.2E-16**
	Endobiotic community	2.6	0.185	**187.2**	**2.2E-16**
	Epibiotic community	2.2	0.231	**275.5**	**2.2E-16**

Bold indicates a significant effect ($P < 0.05$).

Fig. S3). The case of co-occurrence of *D. villosus* and *P. robustoides* in Wisla Sobieszewska revealed significant distinctions in the richness of symbiotic communities only for the epibiotic subset (Supplementary Fig. S4). The two-way GLM analysis for the case report of the native host, *G. zaddachi*, and the American invasive *G. tigrinus*, from the Dźwirzyno Regoujście and Rowy Canal, revealed a significant effect of locality on α richness for the whole and endobiotic communities with no host and interaction effect between host species and locality (Supplementary Tables S4-S6, Supplementary Fig. S8). The α richness of whole symbiotic communities of *D. villosus* from different localities varied slightly (Supplementary Fig. S5A). The highest α richness of the whole symbiotic community in the Mrzeżyno Pera was significantly different from the lowest in Port Lodolamaczy. The α richness of endobiotic and epibiotic communities of *D. villosus* was significantly poorer in the Rowy Canal and Port Lodolamaczy, respectively, compared with other localities (Supplementary Fig. S5B and C). A significantly higher α richness of the whole and epibiotic communities of microorganisms from *G. pulex* was found in Smoldzino Lupawa and Krępa Słupska compared with other localities (Supplementary Fig. S6A and C). The endobiotic community richness was only significantly higher in the Smoldzino Lupawa for *G. pulex* (Supplementary Fig. S6B). The Dźwirzyno Regoujście was the significantly richest locality for whole and endobiotic subsets from *G. tigrinus*, while Martwa Wisla was the significantly poorest locality for epibiotic microorganisms from this host (Supplementary Fig. S7).

4. Discussion

The analysis of community composition of symbiotic microorganisms showed that invasive hosts delivered to the local ecosystems of the Baltic region of Poland at least six co-introduced species: *Cephaloidophora rotunda*, *Cephaloidophora similis*, *Cephaloidophora mucronata*, *Ganymedes ramose*, *Cucumispora dikerogammari* and *Dictyocoela roeselum*. All these species can be regarded as specific to gammarids and have been reported in the host native area of the Ponto-Caspian or Balkan regions (Bălcescu-Codreanu, 1974; Codreanu-Bălcescu, 1995; Ovcharenko et al., 2010; Bacela-Spychalska et al., 2012; Quiles et al., 2020). All of them, except *C. rotunda*, are currently known to infect a number of gammarid species across European waters. Their emergence in the Baltic Region has been attributed to the co-invasion process related to the hosts' movement and occupation of new areas (Ovcharenko et al., 2009, 2010; Bacela-Spychalska et al., 2012; Quiles et al., 2020). Two additional species of gregarines, *Eugregarinorida* gen.sp.1 and *Eugregarinorida* gen.sp.2, found in the invasive *D. villosus* and *G. tigrinus*, are likely co-invaders but further work is needed to clarify their taxonomic identity. These findings conform with our first hypothesis stating that invasive hosts co-introduced symbiotic species to the local ecosystems, thereby expanding the number of potential parasites and pathogens in the Baltic region of Poland. Nevertheless, the native naïve symbiotic microorganisms, ciliates and gregarines, are dominated in the species number among all studied gammarid hosts and in all localities. Hence, based on this observation, we expect that the native symbiotic species will largely play a key role in regulation of the host population dynamics in the region.

The results of the present study provide evidence both for and against the ERH. On the one hand, the highest symbiotic species richness at both α and β levels was reported in the native *G. pulex* and the lowest in invasive hosts (e.g. in *E. ischnus*, *P. robustoides* and *G. tigrinus*) (Table 1 and Fig. 3). On the other hand, the species richness of symbionts in the second native host, *G. zaddachi*, was similar to that of invasive gammarid species, *D. villosus* and *G. roeselii*. The similarity in symbiont richness could be explained by the co-occurrence of the native *G. zaddachi* with the invasive *G. tigrinus* and *D. villosus*. We suppose that the differences in the richness of symbiotic communities between the two native hosts, *G. pulex* and *G. zaddachi*, are largely related to the habitat conditions of these gammarids. Whereas *G. pulex* was sampled only in freshwater lotic habitats, *G. zaddachi* occurred in eutrophicated brackish waters. Both localities, the Rowy Canal and Dźwirzyno Regoujście, inhabited by *G. zaddachi* were characterized by the highest chlorophyll-a level (Mendeley data, https://doi.org/10.17632/c9fzyyzr52.1, File 3). In our case study of the Lupawa system, *G. pulex* was sampled in lotic habitats with relatively high phosphorous content and moderate chlorophyll-a levels. This sample was characterized by a high species richness of symbionts. Five kilometres below, the Lupawa River flows into the Gardno Lake which is connected by a canal to the Baltic Sea. Three gammarid species, *D. villosus*, *G. tigrinus* and *G. zaddachi*, were found in this canal (the Rowy Canal locality) with less diverse symbiotic communities compared with that of *G. pulex* from the upper Lupawa locality (Table 1). It should be noted that both *D. villosus* and *G. tigrinus* were infected with two co-introduced symbiotic species in the Rowy Canal that potentially could increase the richness of the symbiotic community. The algae partially consumed nutrient contents in the Gardno Lake, decreasing the total phosphorous concentration twice and nitrogen 10-fold in the Smoldzino Lupawa compared with the Rowy Canal, while the chlorophyll-a level was substantially increased (Mendeley data, https://doi.org/10.17632/c9fzyyzr52.1, File 3). Moreover, the Rowy Canal locality is characterised by brackish water in contrast to freshwater in the Lupawa locality. The present findings seem to be consistent with other studies, which found the eutrophication (waters with chlorophyll-a level > 5 mg/m³ classed as eutrophic (Darko and
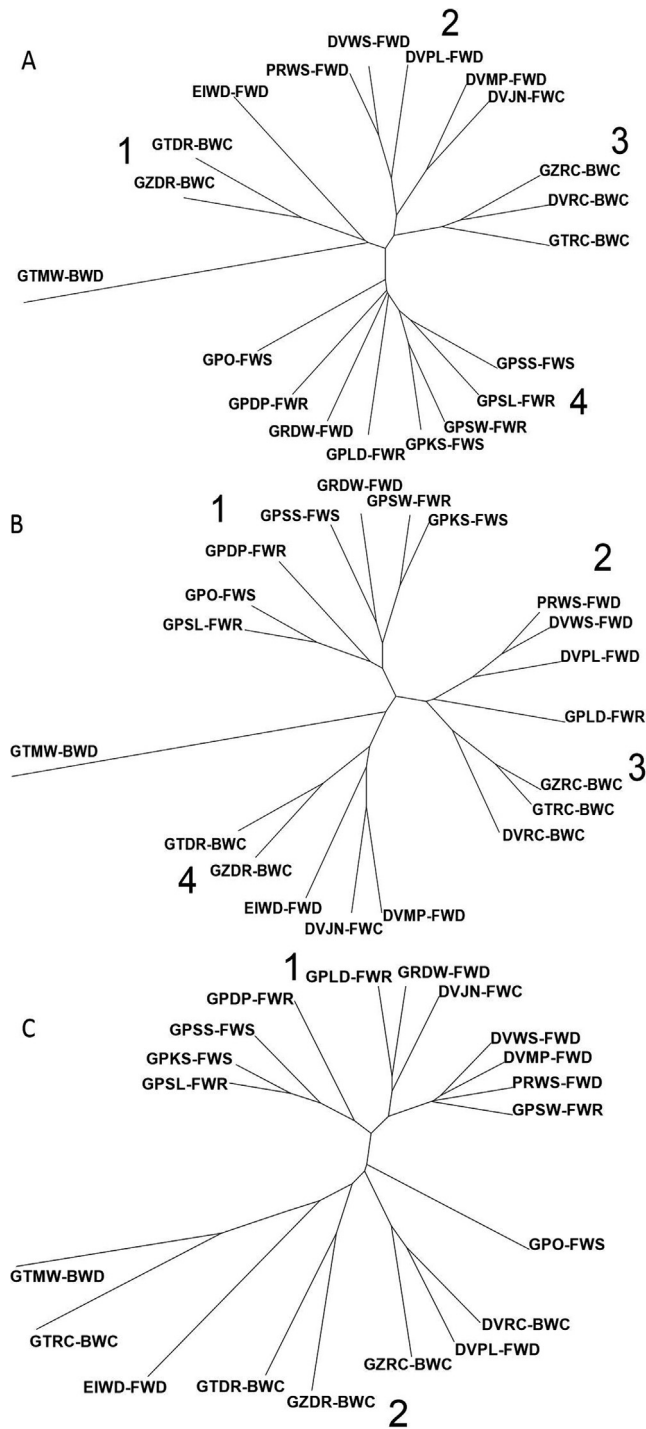
Fig. 4. Clustering pattern of β1 richness of the whole (A), epibiotic (B) and endobiotic (C) communities of all gammarid samples in the Baltic region of Poland with information on the habitat. Dendrograms were constructed using the Whittaker's index of similarity and the unweighted pair-group mean average method. The branches are marked with sample abbreviations denoting the host (the first two letters) and locality names (the third and fourth letters). Key clusters are marked with digits. Sample codes (first 3–4 letters) as defined in Table 1; habitat abbreviations (three letters after the hyphen) are as follows: FWS, freshwater stream; FWR, freshwater river; FWD, freshwater delta; FWC, freshwater canal; BWD, brackish water delta; BWC, brackish water canal.

Ansa-Asare, 2010)) and salting as important factors limiting both host and symbiont diversity of freshwater ecosystems (Budria, 2017; Bommarito et al., 2022).

Our data corroborate previous findings of Wattier et al. (2007), Ovcharenko et al. (2009, 2010), Bacela-Spychalska et al. (2012),

Bojko et al. (2013), Grabner et al. (2015), Quiles et al. (2019), who found no evidence for the absence microsporidian and gregarine parasites in Ponto-Caspian and Balkanic gammarids occurring in sites within the European continental-invasive range. It is therefore likely that this group of invasive amphipods underwent massive or recurrent introductions during the colonisation of Central and Western European waters to move there, their multiple parasites occurring within its native range (Wattier et al., 2007; Bojko et al., 2013). In contrast, a low species richness of symbiotic communities and absence of important co-introduced pathogens (microsporidians and apostomatid ciliates) observed within populations of *G. tigrinus* sampled during our study may instead reflect focal, non-recurrent introductions, leading to a loss of symbiotic fauna during the invasion process. Our results, together with previous studies on pathogens and parasites of amphipods transported across the marine channel or ocean (Bojko et al., 2013; Banha et al., 2018), indicate that open marine waters can serve as a natural barrier preventing the co-introduction of symbiotic organisms. The mechanism of parasite co-introduction in amphipods may depend on the nature of the host translocation. Ponto-Caspian and Balkanic gammarids self-disperse or are shipped short distances along migration corridors via European rivers and connecting canals (Bij de Vaate et al., 2002). They exhibit several symbiotic organisms typical of their native range that have formed self-established populations in Central and Western European waters (Wattier et al., 2007; Ovcharenko et al., 2009, 2010; Bacela-Spychalska et al., 2012; Bojko et al., 2013; Grabner et al., 2015; Quiles et al., 2019). In contrast, in species transported by ballast water over long distances (e.g. across the open sea), pathogen loss has been observed due to either low initial population density of both host and pathogen at the new range, or death of heavily infected hosts with their pathogens during the translocation process (Torchin et al., 2003; Bojko et al., 2013). Thus the described pattern of amphipod dispersion illustrates the stepping-stone mechanism of ERH, where secondary expansion leads to additional reductions in parasite infection through multiple founding events (Chalkowski et al., 2018).

Our results are in accordance with our initial expectation of both locality and host species being drivers of the symbiotic community structure of gammarids, which generally agree with previous studies of other host-parasite systems (Sasal et al., 1997; Llopis-Belenguer et al., 2020b; Moss et al., 2020; McNew et al., 2021). The closest similarity for both α and β species richness of symbionts was found between sympatric host species; in all cases of host co-occurrence the distinction in the richness of symbiotic communities was insignificant and low. Although there was no apparent effect of host species on the richness of symbiotic communities in all revealed cases of host co-occurrence, the ANOVA tests applied to the overall data revealed a significant influence of host species on the α richness. The pairwise analysis showed that the differences are largely related to the highest symbiotic community richness in *G. pulex*. Both α and β richness of symbionts varied between localities for *D. villosus*, *G. tigrinus*, *G. zaddachi* and *G. pulex*, and these differences were significant. This evidence suggests that locality appears to be a stronger determinant of the symbiotic community of gammarids than host species. These results conform with evidence from parasite communities of amphibian hosts in Californian (USA) ponds (Moss et al., 2020), but differ from the host-parasite system of mugilid hosts in the western Mediterranean (Llopis-Belenguer et al., 2020b). The helminth community of mugilids is predominantly composed of host-specific (stenoxenic or oioxenic) species (Sarabeev et al., 2013; Sarabeev, 2015; Sarabeev and Tkach, 2019; Llopis-Belenguer et al., 2020b), while in gammarids the symbiotic community tends to be non-specific (euryxenic). Considering the "filters" theory proposed by Combes (2001) to account for the ability of parasites to colonize hosts,

Table 3
Measuring the dispersion pattern of the α (individual) species richness of symbiotic communities in seven gammarid host species in the Baltic region of Poland by using variance to mean ratio, the Poisson, negative binomial and normal distribution models.

Host	Symbiotic community	Variance to mean ratio	Poisson			Negative binomial			Normal		
			χ²	P	AIC	χ²	P	AIC	W	P	AIC
Gammarus pulex	Whole	0.96	**15.4**	**0.284**	**127.7**[a]	**15.7**	**0.201**	**1029.7**	0.96	8.87E-05	1036.1
	Endobiotic	0.83	14.6	0.012	701[a]	14.8	0.005	703.4	0.9	7.69E-11	720.9
	Epibiotic	1.02	**17.5**	**0.093**	**961.1**[a]	**17.52**	**0.063**	**963.1**	0.95	3.12E-07	975.9
Dikerogammarus villosus	Whole	0.71	28.9	0.0002	612.3[a]	29.5	4.92E-05	614.3	0.96	9.00E-04	602.7
	Endobiotic	0.90	**6.2**	**0.104**	**404.9**[a]	6.3	0.0418	406.8	0.85	1.03E-10	427.1
	Epibiotic	0.69	15.4	0.031	563.9[a]	16	0.014	565.9	0.96	3.00E-04	554.7
Gammarus tigrinus	Whole	0.88	**5.6**	**0.465**	**318.6**[a]	**5.74**	**0.332**	**320.6**	0.94	1.80E-03	323.5
	Endobiotic	0.66	13.2	0.0003	198.6[a]	13.3	0	200.6	0.8	2.05E-09	198.5
	Epibiotic	0.71	10.8	0.029	273.1[a]	10.9	0.012	275.1	0.9	2.57E-05	274.1
Gammarus zaddachi	Whole	0.70	**10.1**	**0.119**	**326.6**	**10.5**	**0.062**	**328.6**	0.96	6.00E-03	322.1[a]
	Endobiotic	0.95	10.5	0.005	231.6[a]	10.7	0.001	233.6	0.85	7.55E-08	239.2
	Epibiotic	0.81	16.3	0.006	298.3[a]	16.5	0.002	300.3	0.88	1.38E-06	306.4
Gammarus roeselii	Whole	1.44	18.7	0.027	150[a]	16.9	0.03	150.3	**0.94**	**0.106**	**153.2**
	Endobiotic	0.60	9.4	0.024	NA	9.5	0.009	NA	0.83	4.00E-04	86.4
	Epibiotic	1.99	**15.6**	**0.112**	**156.2**	**7**	**0.635**	**149.6**[a]	**0.94**	**0.095**	**153.9**
Pontogammarus robustoides	Whole	0.88	**8.2**	**0.224**	**149.9**[a]	**8.4**	**0.136**	**151.9**	**0.95**	**0.093**	**151.7**
	Endobiotic	0.97	**4**	**0.258**	**103.1**[a]	**4**	**0.131**	**105.1**	0.84	8.25E-05	111.7
	Epibiotic	0.75	**4.1**	**0.389**	**129.5**[a]	**4.2**	**0.241**	**131.5**	0.93	0.015	131.5
Echinogammarus ischnus	Whole	0.65	**7.3**	**0.119**	**92.3**[a]	**7.4**	**0.059**	**94.3**	**0.93**	**0.124**	**92.6**
	Endobiotic	0.88	0.7	NA	NA	0.68	NA	NA	0.44	1.11E-08	24.8
	Epibiotic	0.51	**8.6**	**0.07**	**88.4**	8.8	0.032	90.4	**0.93**	**0.0833**	**85.3**[a]

Bold indicates cases when empirical data fit the respective theoretic distribution.
[a] The best model as defined according to the lowest Akaike information criterion (AIC) value.

the host specificity filter seems to be a less important driver of symbiotic communities in gammarids than in mugilid hosts. In other words, "the compatibility filter" is more broadly open for symbiotic species in gammarid hosts.

In addition to geographic locality and host species, habitat condition was also a key determinant of species richness. Distinctions between freshwater and brackish environments were significant for both levels of richness. Within the regional scale of Pomerania, geographic distance was less important than habitat conditions of the locality for shaping symbiont communities of gammarids. The overlap in species composition between the most distant localities was considerable, while it was less evident in localities characterized by different habitat conditions. The most obvious example is a highly distant β richness of symbiont communities between the Martwa Wisla sample (Fig. 4) characterized by the highest salinity level (Mendeley data, https://doi.org/10.17632/c9fzyyzr52.1, File 3) and the close freshwater localities of Wisla Sobieszewska and Port Lodolamaczy. Moreover, samples from streams and rivers located along longitude gradients were clustered together on the distance trees based on the Whittaker's index of species composition similarity, while geographically close brackish localities were out of this clade (Fig. 4A). A similar pattern was observed in the endobiotic subset of the community data to group symbiotic communities from localities with brackish water on the tree (Fig. 4C). These results suggest environmental filtering of parasite diversity, which is consistent with previous findings reporting habitat effects

on parasite communities in aquatic and terrestrial environments (e.g. Krasnov et al., 2015; Kołodziej-Sobocińska, 2019; Levy et al., 2019; Llopis-Belenguer et al., 2020b).

In most cases, the dispersion pattern of species richness showed the best fit to the Poisson distribution model (Table 3). This suggests that the symbiotic community found at the individual host level is a random subset of a large group of parasite species within a host species or locality (Poulin, 1996; Llopis-Belenguer et al., 2020b). It also highlights that symbiotic assemblages are more likely to be the product of random events rather than predictable and repeatable processes (Poulin, 1996). The unique exception to this general rule concerned species richness of epibiotic symbionts of the Balkan invader *G. roeselii*, which exhibited an overdispersed distribution (based on variance to mean ratio), fitted best by a negative binomial distribution. Both *G. pulex* and *G. roeselii* showed the highest α richness of symbiotic community in the studied area but the dispersion pattern of their epibiotic communities was different, random versus overdispersed (Table 3). The density plot of the distribution of epibiotic communities from *G. roeselii* was right-skewed, illustrating a lower proportion of the host population being infected with a large number of symbiotic species compared with *G. pulex* (Supplementary Fig. S1B). *Gammarus roeselii* and native symbiotic species lack coevolutionary adaptations. Thus compared with the system of *G. pulex* and its symbiotic community, it is more likely that the survival rate of microorganisms in *G. roeselii* is lower, on one hand, and rescue of this host against

infection is higher, on the other hand. As a result of this complex process, the mode of frequency distribution shifted to the low values of species number per infected host determining a relatively long distribution tail. This result fully corroborates our earlier findings on the host-parasite system of grey mullet fish and helminth parasites which reported a similar dispersion pattern of individual species richness in native and invasive hosts (Sarabeev, 2015; Sarabeev et al., 2022a). However, we should note that the right-skewed distribution of α richness was reported only in rich species assemblages of invasive hosts, while the species with poorer symbiotic communities from invasive gammarids showed typical random dispersion.

We believe this is the first analysis of the symbiotic species richness in native and invasive gammarid hosts in European waters based on original field data of the broad range of endobiotic and epibiotic organisms representing nine phyla to document the patterns of species composition and distribution. Furthermore, the taxonomically diverse complex of species representing nine major protozoan and metazoan higher taxa and differing infection sites allowed us to assess the effect of host translocation and regional ecological determinants driving assembly richness in the model aquatic symbiotic systems studied. Our study reveals that (i) the current assemblages of symbionts of gammarid hosts in the Baltic region are formed by native and co-introduced species; (ii) species richness of the symbiotic community was higher in the native *G. pulex* than in invasive hosts, probably reflecting a process of species loss by invasive gammarids in the new area and distinct habitat conditions occupied by *G. pulex* and invasive hosts; (iii) both host species and locality are key drivers shaping assembly composition of symbionts, whereas habitat condition was a stronger determinant of communities than geographic distance; (iv) the dispersion patterns of the individual species richness of symbiotic communities were best described by Poisson distributions; in the case of an invasive host, the dispersion of the rich species diversity may switch to a right-skewed negative binomial distribution, illustrating a host-mediated regulation process. Further research will focus on assessing abiotic and biotic factors shaping infection parameters and species assemblages of symbionts, and study their dispersion patterns and host-parasite relationships.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2023.02.006.

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