



Distribution of different species of metacercariae in two freshwater fishes: *Haludaria fasciata* (Teleostei: Cyprinidae) and *Pseudosphromenus cupanus* (Teleostei: Osphromenidae)

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Abstract Information on the distribution and abundance patterns of trematodes are essential to reveal the ecology of host–parasite interactions. The Western Ghats of India, a biodiversity hotspot, is rich in freshwater fish diversity and endemism. Though there are several studies on various other aspects of fish ecology, studies on their parasitic fauna is meager. The objective of the present study is to explore the distribution and infection patterns of metacercariae of five species of trematodes in the freshwater fishes, *Haludaria fasciata* and *Pseudosphromenus cupanus*. The infection parameters were analyzed for each host and CART model was applied to analyze the environmental factors affecting parasite distribution patterns. All species of metacercariae showed an over-dispersed aggregate distributions. The classification tree models indicated that among the environmental factors considered, differences in host locality was the most influential factor in both fishes, followed at a greater distance by the factor seasonality. The parasite communities exhibited temporal and spatial differences in the infection pattern in response to seasonal and locational variations.

Keywords Metacercariae · Aggregation · CART model · *Haludaria fasciata* · *Pseudosphromenus cupanus*

Introduction

Freshwater fishes are hosts to taxonomically diverse trematode parasites as both intermediate and definitive hosts. In the complex life cycles of helminth parasites, intermediate hosts often serve mainly as vehicles whose purpose is to take the parasite to their definitive hosts (Lafferty 1999). These parasite communities exhibit temporal and spatial changes in response to seasonal and locational variations in several biotic and abiotic environmental factors; these variations can be reflected in parasite species composition (Holmes 1987; Poulin 1998; Thieltges et al. 2011). The Western Ghats region of India, one of the biodiversity hotspots, is rich in freshwater fish diversity and endemism (Dahanukar et al. 2004, 2011; Raghaven et al. 2013). The melon barb, *Haludaria fasciata* (Jerdon, 1849) is an endemic freshwater fish inhabiting the rivers flowing through the Western Ghats (Chakraborty et al. 2017) and the spike tail paradise fish, *Pseudosphromenus cupanus* (Cuvier, 1831) contributes to the thriving aquarium industry of the Western Ghats regions (Raghaven et al. 2013).

It is essential to explore the parasite fauna and their population dynamics to reveal their effects on hosts such as: (a) production of a metabolic cost using host resources; (b) increasing the probability of mortality due to direct serious pathology, either by favoring new parasitic infections or by weakening the organism; (c) decreasing the reproductive rate and (d) causing changes in host behavior, including migratory movements (Mangel 2006; Poulin et al. 2012). Furthermore, knowledge on the ecological role of parasites and their biodiversity are essential to understand the functioning of any ecosystem. Essential data on the knowledge of the population parameters of parasites, in

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the host-parasite systems, as well as their spatial and temporal characteristics are scanty.

Study on the distribution and abundance patterns in nature and the causes and factors that determine them occupies a central role in the science of ecology (MacArthur 1972; Kingsland 1995; Krebs 2001). Knowledge on the shape of the frequency distributions of the variables under study in ecology not only serves as a statistical or phenomenological description of an event or model, but also informs us about the type of processes that cause it (Marquet 2009). In this way, the essential information that explains the range and the spatial pattern of distribution, the environmental factors that determine them, the dynamics of populations (e.g. of parasites and their hosts), epidemiological processes in the case of parasites, driving forces in evolutionary ecology, and finally the sampling strategy in data collection can be obtained (Li and Hsü 1951; Anderson and May 1978; Lester 1984; Krebs 1989; Schmidt and Roberts 2000; Southwood and Henderson 2000; Rosa and Puglieses 2002).

Parasites in general show an aggregate distribution among their hosts, so that heterogeneity in the number of parasites per host is the norm rather than the exception (Crofton 1971; Shaw and Dobson 1995; Poulin 2007). These distribution patterns are characterized by presenting statistical distributions with only positive values (count data), clearly skewed to the right, having a variance greater than the mean and the latter being non-constant, increasing as the mean value becomes greater (Shaw and Dobson 1995; Poulin and Morand 2000). One of the statistical distributions that best fits these characteristics is the negative binomial distribution, which has been widely used to represent over-dispersion and describe the distribution patterns of parasites (Poulin 2007). All these features are counterproductive for the application of classical linear statistical models based on the normal distribution (Dunn and Smyth 2018). Among the most used methods to describe and to model the infection patterns of parasites is the application of generalized linear models, GLMs, (McCullagh and Nelder 1989; Wilson and Grenfell 1997; Alexander 2012) and their extension when there is an abundance of zeros: the zero inflated models (O'hara and Kotze 2010; Chipeta et al. 2014; Rhodes 2015). However, when studying the influence of environmental or other factors on the levels of infestation observed, the variable selection process can be difficult and model-specific (Qian 2017). The classification and regression tree models (CART Breiman et al. 1984) and their multivariate extension (De'Ath 2002) are very useful for identifying variables that contribute to the response variable and for prediction and classification (De'Ath 2002; Qian 2017). Therefore, CART models have been applied successfully in various fields of ecology (De'Ath and Fabricius 2000; Roff 2006)

including the studies on the influence of environmental factors on parasite infection levels (Vignon and Sasal 2010; Gazzinelli et al. 2017; Rossiter and Davidson 2018).

In the present study, the main objectives were to know the distribution pattern and the infection parameters of different species of metacercariae in *H. fasciata* and *P. cupanus*. CART models were used for study the influence of environmental factors such as the seasonality and difference in locality, on the infection patterns of metacercariae of melon barb and spike tail paradise fish. Special emphasis was given to the trematodes that showed a greater presence, such as *Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936, *Haplorchoides mehrai* Pande and Shukla, 1976 and *Tetracotyle wayanadensis* Jithila and Prasad, 2018 in *H. fasciata* and *A. burminis* and *Clinostomum complanatum* (Rudolphi, 1819) in *P. cupanus*.

Materials and methods

Study area and sampling of fishes

The Western Ghats of India with its geographical extension in the wet zone of Sri Lanka are considered as a biodiversity hotspot owing to its rich biodiversity and concentration of endemism. Ninety two specimens of *H. fasciata* and 106 specimens of *P. cupanus*, were collected from water bodies in different localities of the Wayanad region (lies between North 11'27' and 15'58' and East 75'47' and 70'27') of the Western Ghats (Fig. 1) during March 2017 to February 2018.

Collection of metacercariae

The collected *H. fasciata* and *P. cupanus* were brought alive to the laboratory and maintained in clean glass aquariums. Fishes were fed occasionally with fish meal. The scales, skin, gills, gill chambers and eyes of sacrificed fishes were examined under Labomed (Luxeo 4Z) stereozoom microscope for larval digeneans. Skin was removed, and the muscle tissues macerated to detect the metacercariae, if any. Internal organs were dissected out and placed in separate petri-dishes containing 0.75% saline, macerated and examined under the stereozoom microscope. The metacercariae of *A. burminis*, *H. mehrai*, *T. wayanadensis*, *C. complanatum* and *Diplostomum ketupanense* Vidyarthi, 1937 collected from *H. fasciata* and *A. burminis*, *H. mehrai* and *C. complanatum* from *P. cupanus* were carefully transferred to 0.75% saline in a petri-dish. Larvae were excysted either by rupturing the cyst wall with fine needles or by mounting them under cover glass and applying gentle pressure over it by fine needles. The excysted larvae were studied under a Nikon ECLIPSE Ni-U phase contrast

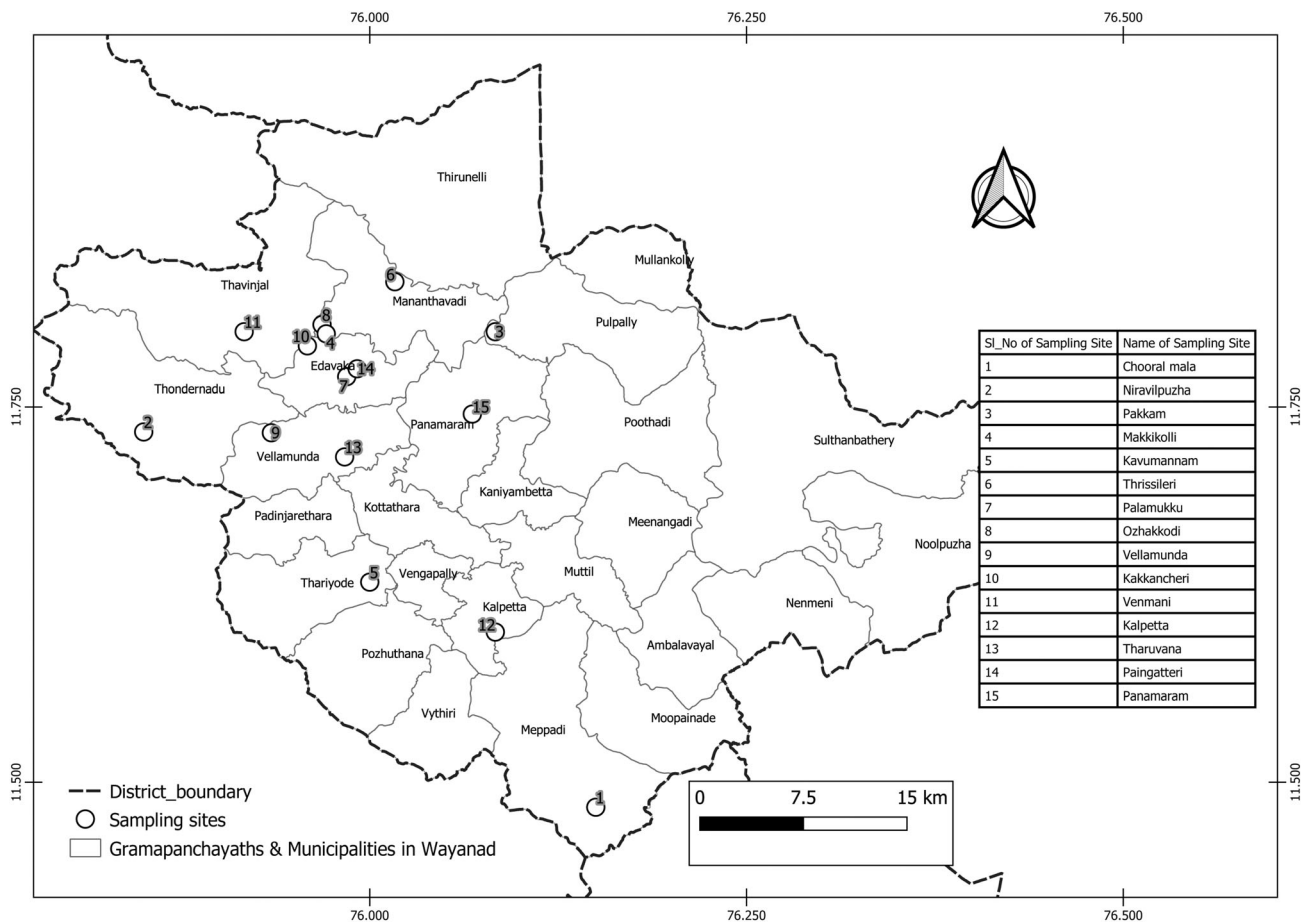


Fig. 1 Study area. Locations of specimen collection in the Wayanad region

research microscope (Japan) without stains or with vital stains like neutral red.

Quantitative analysis

For the study of infection patterns, samples collected during one year were considered. The analyses were performed using the data on host species and that of metacercariae obtained. Only the metacercariae obtained from more than one host specimen were considered for analyses, i.e. metacercariae of *A. burminis*, *H. mehrai* and *T. wayanadensis* of *H. fasciata* and *A. burminis* and *C. complanatum* of *P. cupanus*. Metacercariae of *C. complanatum* and *D. ketupanense* were obtained only from one specimen of *H. fasciata*, and therefore, their presence only was recorded. Similarly the presence of *H. mehrai* in *P. cupanus* was recorded.

Histograms were used to represent the distribution of the number of parasites per host, where the abundance of zeros and the skewed to the right were discerned. The infection parameters analyzed for each host and for the aforementioned parasites were: prevalence, mean intensity, median

intensity and mean abundance (Bush et al. 1997; Reiczigel et al. 2019). In addition, the following aggregation indices were also analyzed: variance to mean ratio, Poulin’s discrepancy index *D* and the parameter *k* of the negative binomial distribution (Wilson et al. 2002; Poulin 2007). These analyses were carried out following the methodology described by Reiczigel et al. (2019), using the free software accessible on the Quantitative Parasitology Web portal (<http://www2.univet.hu/qpweb/qp10/index.php>).

The levels of over-dispersion of different metacercariae were analyzed using the graphs of relationship of log mean abundance versus log mean variance. The negative binomial distribution, suitable to describe cases of over-dispersion in discrete variables where the variance is greater than the mean (Bolker 2008), which normally takes place in the distribution of parasites, was analyzed. The adjustment of the negative binomial distribution to the observed distribution of the trematodes per hosts was made using the R package ‘fistdistrplus’ (v. 1.1–1, Delignette-Muller et al. 2014). Goodness of fit was measured through psi squared tests carried out using the Quantitative Parasitology Web Portal above mentioned.

Table 1 Quantitative descriptors of metacercariae infection in *Haludaria fasciata*

Parasite species	<i>Acanthostomum burminis</i>	<i>Haplorchooides mehrai</i>	<i>Tetracotyle wayanadensis</i>	<i>Clinostomum complanatum</i>	<i>Diplostomum ketupanense</i>
Prevalence % Confidence Interval	48.9 (38.3–59.6%)	19.6 (12.0–29.1%)	6.5 (2.4–13.7%)	1.09	1.09
Mean Intensity Confidence Interval	5.04 (4.13–6.13)	8.44 (5.06–13.9)	2.83 (1–4)	3.00	3.00
Range	1–14	1–32	1–5		
Median Intensity Confidence Interval	5 (3–6)*	4 (2–5)**	2.5F (1–5)***		
Mean Abundance Confidence Interval	2.47 (1.84–3.21)	1.65 (0.859–3.2)	0.185 (0.0543–0.402)	0.03	0.03
Range	0–14	0–32	0–5	0–3	0–3
Aggregation index: Variance to mean ratio	4.89	16.51	3.92		
Aggregation index: Poulin's discrepancy D	0.686	0.894	0.947		
Confidence Interval	(0.619–0.753)	(0.845–0.937)	(0.904–0.975)		
Aggregation index: <i>k</i> parameter from NBD(+) ML estimate (++)	0.3469 Fit to NBD is acceptable $P = 0.2717$	0.0694 Fit to NBD is acceptable $P = 0.2316$	Sample too small (of infected fish) to check NBD		

* Actual (exact) confidence level: 95.6%; ** Actual (exact) confidence level: 95.2%; *** Actual (exact) confidence level: 96.9%; (+) Negative Binomial Distribution; (++) Maximum Likelihood Estimate

Analyses of environmental factors on the distribution of parasites

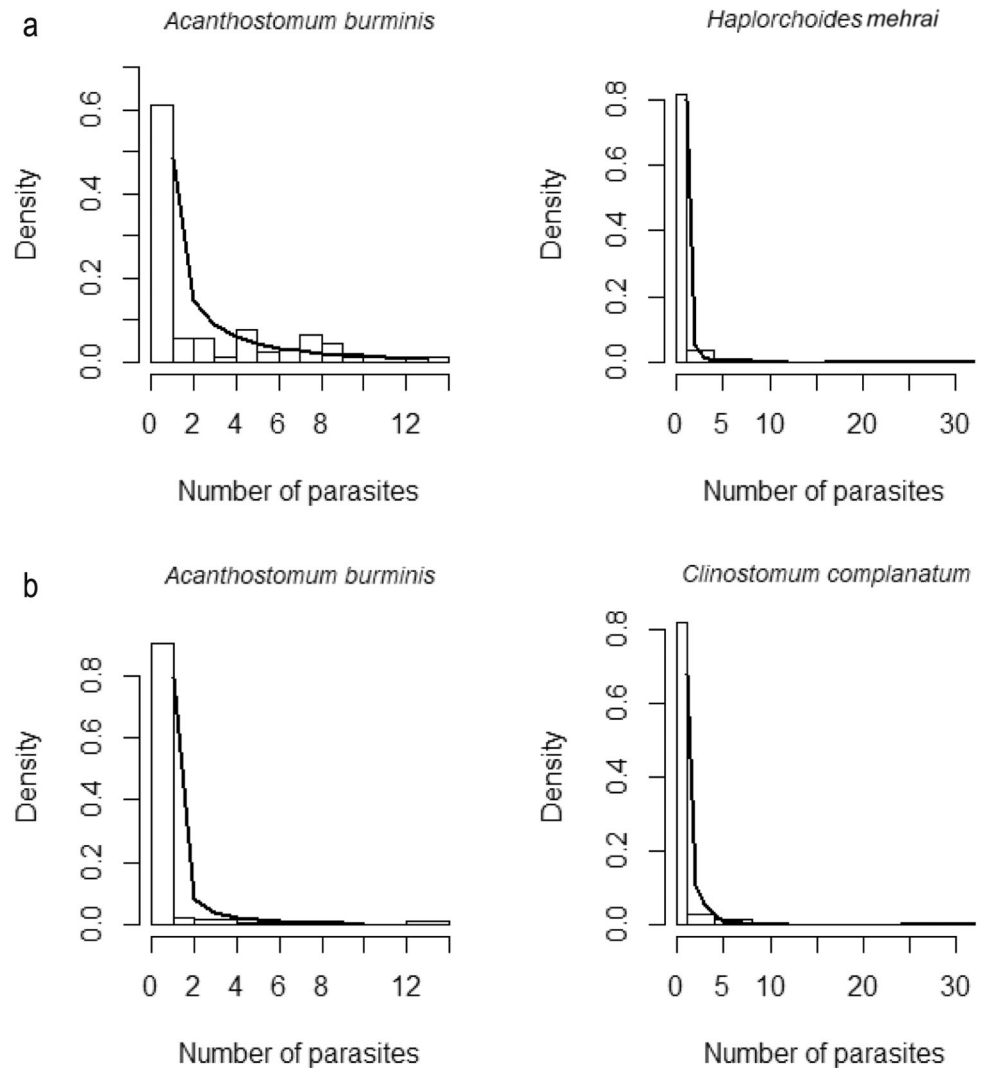
Influence of seasonal period (winter, summer, monsoon and post monsoon) and difference in collection localities were examined. To analyze their contribution on parasitic distribution, parasites having five or more sampled hosts only were considered. Boxplot was employed to explore the possible influence of seasonality and difference in locality on the distribution of parasites per host, especially when two or more samples were compared (Sokal and Rohlf 1995). Graphs for each factor in relation to each trematode species were constructed and used as exploratory tool.

CART model was applied to analyze the environmental factors affecting parasite distribution patterns. CART models are nonparametric statistical methods based on an algorithm known as recursive partitioning (Breiman et al. 1984). It was a step-by-step process by which a decision tree was constructed by either splitting or not splitting each node on the tree into two daughter nodes—a binary split (Izenman 2008). In the classification tree model the output variable was a binary-valued or discrete in the distribution of parasites. This process tends to produce over fitting, so for remedy it a pruning system was used based on the smallest prediction error obtained in a cross-validation procedure (Kabacoff 2015). The R package 'rpart' (v

4.1.15, Therneau et al. 2019) was used for the application of CART models. Following the structure of the R package rpart, the formula, Variable Y (parasites per host) ~ Factors (Season + Place + Type of water course) was used. Among the arguments of the formula, the definition of the “method” was “Poisson”. This method was used when the variable Y (distribution of the number of parasites per host) is a vector or a two-column matrix. If it is a matrix, the first column was the observation times and the second was counts. Other argument referred to the “control parameters” was the complexity parameter. This parameter was set at 0.001, which implies that the split must decrease the overall lack of fit by a factor of 0.001 before being attempted. Therefore, it gave an indication of the most appropriate number of splits in the classification tree analysis. In the same way the program results showed the values of cross-validation with an indication of the error and also the percentage explanation of total variance in each split. Those information were used to define the appropriate number of splits in the model. The plots of classification tree models were obtained using the R package “rpart.plot” (v 3.0.8, Milborrow 2018).

Along with the use of software of the quantitative parasitology web portal, the rest of the statistical analyses and all the figures were made using R statistical software (R Core Team 2020 v 3.6.3).

Fig. 2 **a** Density plots comparing the density function of the fitted negative binomial distribution (solid line) along with the histograms, on a density scale, of the empirical distribution of metacercariae (columns) in *Haludaria fasciata* and **b** in *Pseudosphromenus cupanus*



Results

Distribution patterns

All species of metacercariae showed over-dispersed distributions with the variance greater than the mean (Table 1) and high abundance of zeros in both host fishes, *H. fasciata* and *P. cupanus*. In *H. fasciata*, the negative binomial distribution fits acceptably to the distributions of the number of parasites per host of the metacercariae *A. burminis* (Table 1, X^2 , $p = 0.2717$) and *H. mehrai* (Table 1, X^2 , $p = 0.2316$; Fig. 2a). In the case of *T. wayanadensis* the sample of infected fish was too small to check for negative binomial distribution. In *P. cupanus*, the negative binomial distribution fits acceptably with the distribution of the number of parasites per host in *A. burminis* (Table 2, X^2 , $p = 0.1159$) and *C. complanatum* (Table 2, X^2 , $p = 0.1127$; Fig. 2b).

Infection statistics and indices of aggregation

The metacercariae which showed the highest prevalence were *A. burminis* in *H. fasciata* (48.9%) and *C. complanatum* in *P. cupanus* (33%) (Table 1 and 2). Despite the relatively low prevalence of *H. mehrai* in *H. fasciata* (19.6%), its mean intensity was remarkable (8.44) as well as the range (1–32). In the case of remaining metacercariae, the average intensity barely exceeds the value of five as maximum, although the range was very wide in *C. complanatum* infection in *P. cupanus* (1–29). Mean abundance of infection was low for all parasite species observed in both hosts (Tables 1 and 2).

The variance to mean ratio was greater than one for all metacercariae infected in both *H. fasciata* and *P. cupanus*, confirming an over-dispersed (aggregated) distribution of these parasites. The metacercariae showed indices D values corresponded to aggregate distributions was higher than

Table 2 Quantitative descriptors of metacercariae infection in *Pseudosphromenus cupanus*

Parasite species	<i>Acanthostomum burminis</i>	<i>Haplorchoides mehrai</i>	<i>Clinostomum complanatum</i>
Prevalence % Confidence Interval	20.8 (13.5–29.7%)	0.94	33.0 (24.2–42.8%)
Mean Intensity Confidence Interval	3.45 (2.18–5.73)	4.00	4.2 (2.66–7.23)
Range	1–14		1–29
Median Intensity Confidence Interval	1 (1–3)*		2 (1–3)**
Mean Abundance Confidence Interval	0.717 (0.371–1.32)	0.04	1.39 (0.821–2.49)
Range	0–14		0–29
Aggregation index: <i>Variance to mean ratio</i>	7.35		11.85
Aggregation index: <i>Poulin's discrepancy D</i>	0.894		0.85
Confidence Interval	(0.854–0.93)		(0.793–0.9)
Aggregation index:	0.1159		0.179
<i>k</i> parameter from NBD(+) ML estimate (++)	Fit to NBD is acceptable $P = 0.4625$		Fit to NBD is acceptable $P = 0.58$

*Actual (exact) confidence level: 97.4%; ** Actual (exact) confidence level: 95.5%

(+) Negative Binomial Distribution; (++) Maximum Likelihood Estimate

0.85 in all cases, except *A. burminis* infection in *H. fasciata* which reached a value of 0.686. The *k* values obtained for the metacercarial infection in *H. fasciata* and *P. cupanus* were low and none exceeds the value of 0.4. The *k* with the lowest value was found for *H. mehrai* infection in *H. fasciata* (Tables 1 and 2).

Influence of environmental factors on distribution of metacercariae

Boxplots showed that seasonality had influence on the distribution of the number of parasites per host in the case of *A. burminis* in *H. fasciata* and *P. cupanus* (Fig. 3a, b) and *C. complanatum* infection in *P. cupanus* (Fig. 3c). Likewise, the distribution of the number of parasites per host was inclined by difference in locality in *A. burminis* and *H. mehrai* infection in *H. fasciata* (Fig. 3d, e) and to a lesser extent *C. complanatum* infection in *P. cupanus* (Fig. 3f).

The classification tree models indicated that among the environmental factors considered, the difference in locality was most influential on distribution of parasites in both *H. fasciata* and *P. cupanus*, followed at a great distance by the factor seasonality. In the case of *A. burminis* infection in *H. fasciata*, the classification tree model resulted in four splits explaining the 58.6% of the total variability. The first split, based on the locality which explained 44% of the variability and the second split, based on seasonality with 8.6% of variability (Fig. 4). The infected fishes were more

abundant in Kalpetta, Makkikolli and Venmani and with higher mean abundance during the summer season. The distribution pattern of *H. mehrai* in *H. fasciata* was also influenced principally by the difference in locality with Makkikolli and Palamukku showed highest infestation levels (Fig. 3e). In the case of *C. complanatum* infection in *P. cupanus* the classification tree model resulted in three splits explaining the 47.7% of the total variability mainly based on locality and seasonality factors. The first split, based on locality which explained 34.8% of the variability and the second split, seasonality, explained the 11.4% of the total variability (Fig. 5). The infected fishes were more abundant in Kakkancheri and Tharuvana with higher mean abundance during the monsoon season.

Discussion

Over dispersed distributions of helminth parasites are commonly observed within host populations (Shaw and Dobson 1995; Shaw et al. 1998) and are important for understanding many density-dependent processes in host-parasite interactions (Anderson and May 1978; Keymer 1982). The proximate causes of such aggregated or clumped frequency distributions are poorly understood, especially from natural systems (May and Southwood 1990; Jaenike 1994), but heterogeneity among hosts in the exposure to infective parasite stages is thought to be one important factor (Crofton 1971).

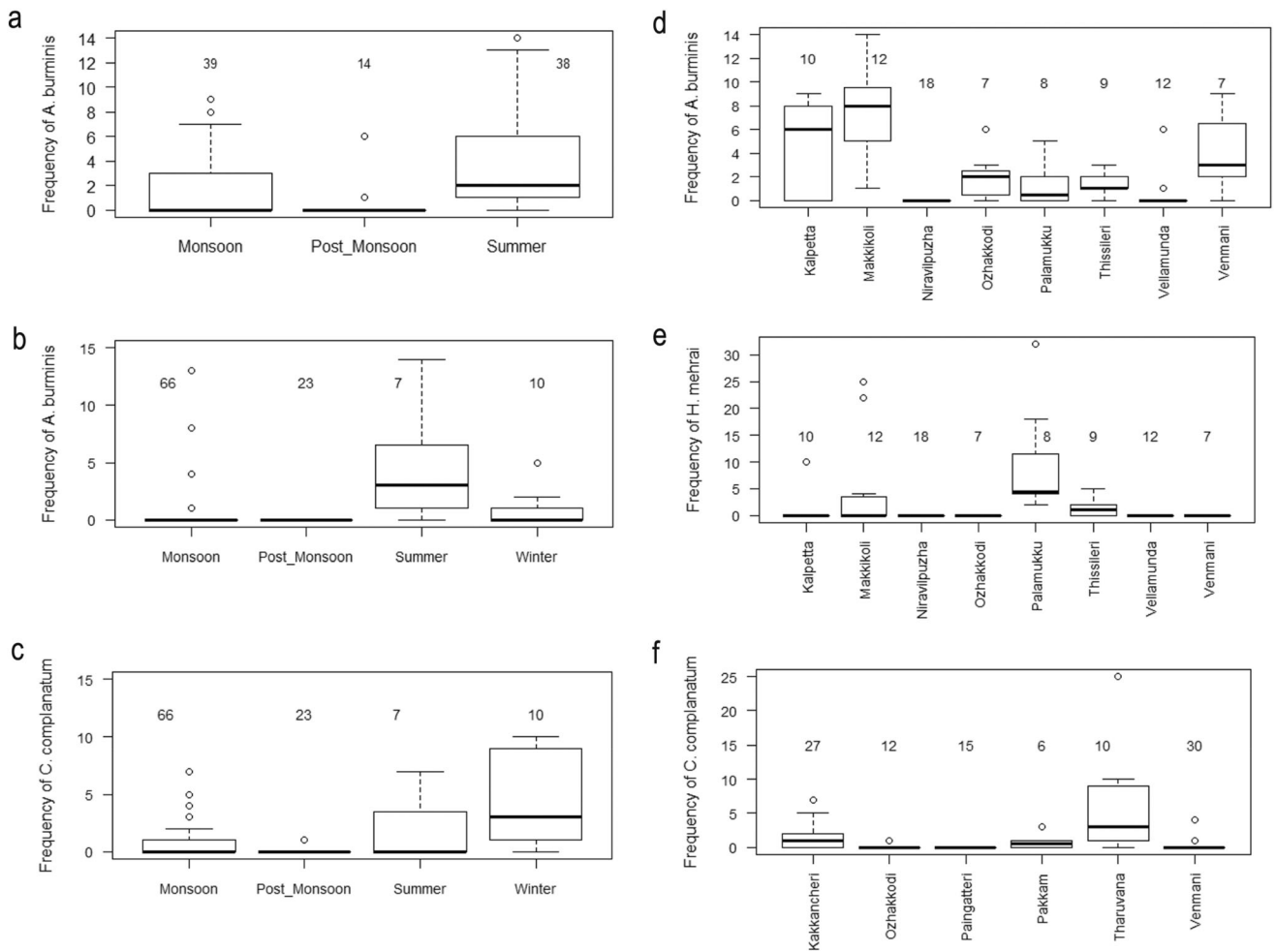


Fig. 3 The frequency distribution of *Acanthostomum burminis* against the factor variable season in *Haludaria fasciata* (a) and in *Pseudosphromenus cupanus* (b). c Frequency distribution of *Clinostomum complanatum* against the factor variable season in *Pseudosphromenus cupanus*. Frequency distribution of *Acanthostomum*

burminis (d) and *Haplorchoides mehrai* (e) against the factor variable localities in *Haludaria fasciata*. Distribution of *Clinostomum complanatum* against the factor variable localities in *Pseudosphromenus cupanus* (f). Number of fish sampled was indicated on each boxplot

Fig. 4 Classification tree plot of the distribution of the numbers of *Acanthostomum burminis* in *Haludaria fasciata* in consideration with the environmental factors. The nodes include the name of the factor. In the terminal nodes the number of parasites with the number of host samples and the percentage were showed

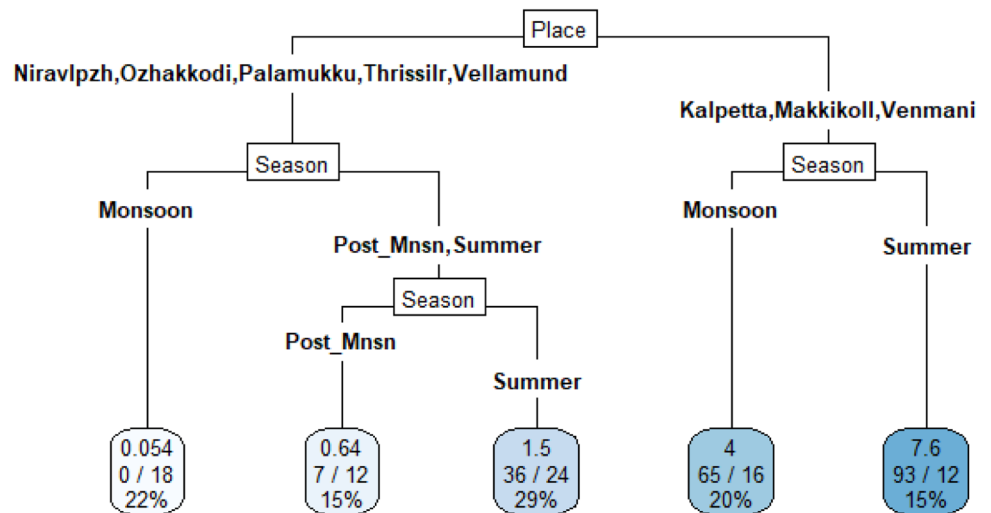


Fig. 5 Classification tree plot of the distribution of the numbers of *Clinostomum complanatum* in *Pseudosphromenus cupanus* taking in consideration the environmental factors. The nodes include the name of the factor. In the terminal nodes the number of parasites with the number of host samples and the percentage were showed

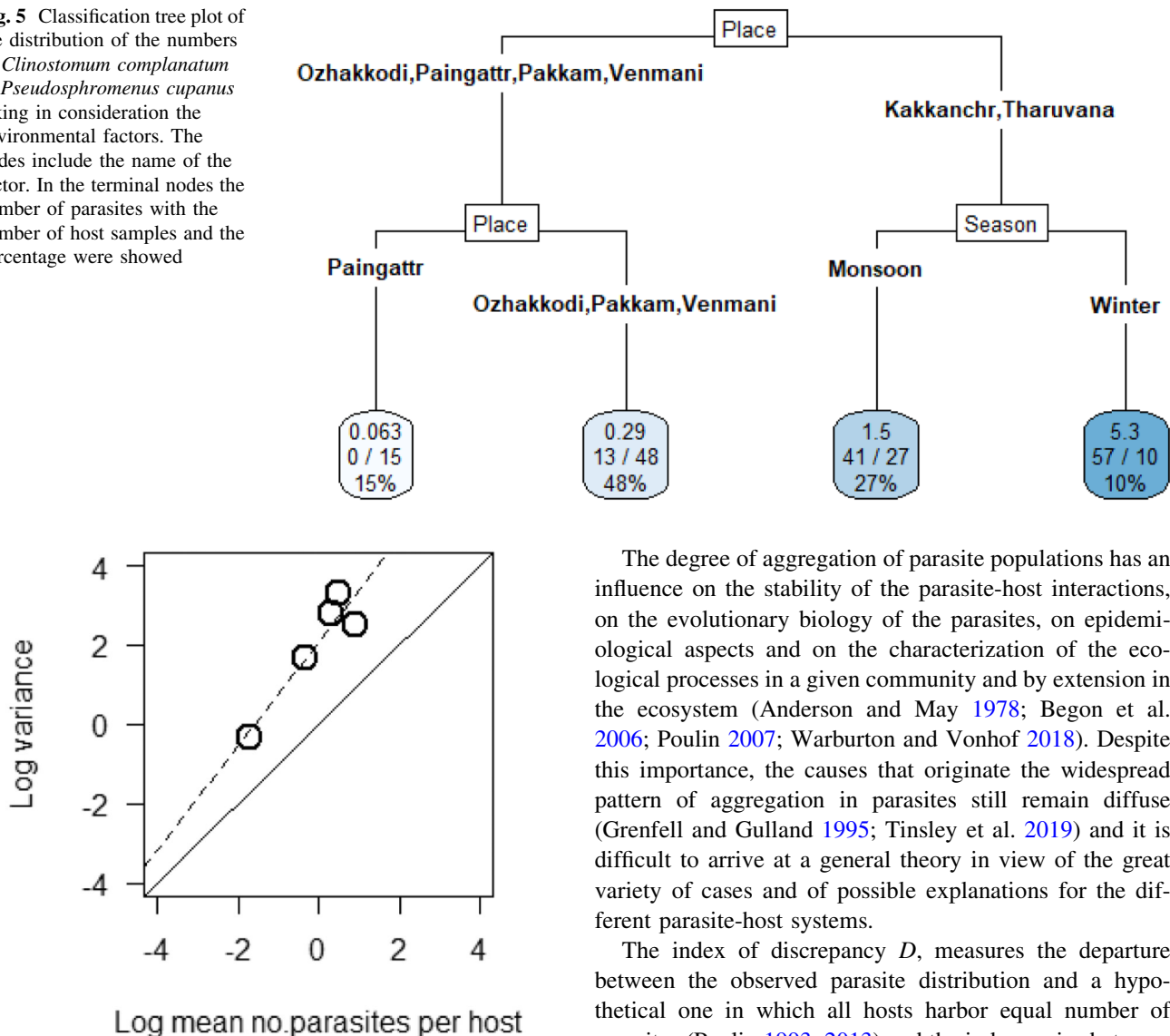


Fig. 6 Relationship between the variance (in log) and the mean number of parasites per host (in log) across the trematode parasite populations infecting *Haludaria fasciata* and *Pseudosphromenus cupanus*. The dashed line is the fitted regression line ($\log \text{variance} = 2.0624 + 1.3084 * \log \text{mean}$; $r^2 = 0.87$, adjusted $r^2 = 0.83$ and s.e. of the slope = 0.2903). The solid line represents the 1:1 relationship above which parasites are aggregated

In the present study the infection of metacercariae in hosts showed over dispersed distribution, which confirms the general pattern of over dispersed distribution characteristic to the parasites (Crofton 1971; Shaw and Dobson 1995). When the data showed over dispersion or a certain degree of extra variation with respect to a Poisson distribution, it implies that the infection rate is not homogeneous in the population (Biggeri 2005). This is essential to understand the causes and consequences of the spatial distribution of parasites (Cohen et al. 2016).

The degree of aggregation of parasite populations has an influence on the stability of the parasite-host interactions, on the evolutionary biology of the parasites, on epidemiological aspects and on the characterization of the ecological processes in a given community and by extension in the ecosystem (Anderson and May 1978; Begon et al. 2006; Poulin 2007; Warburton and Vonhof 2018). Despite this importance, the causes that originate the widespread pattern of aggregation in parasites still remain diffuse (Grenfell and Gulland 1995; Tinsley et al. 2019) and it is difficult to arrive at a general theory in view of the great variety of cases and of possible explanations for the different parasite-host systems.

The index of discrepancy D , measures the departure between the observed parasite distribution and a hypothetical one in which all hosts harbor equal number of parasites (Poulin 1993, 2013) and the index varies between 0 (no aggregation) and 1 (maximum aggregation). The metacercariae showed indices D values, which corresponded to aggregate distributions, higher than 0.85 in all cases, except *A. burminis* infection in *H. fasciata*.

According to Poulin (2013) the heterogeneity in the infection levels in the hosts can be explained based on two broad criteria: the heterogeneity between the hosts in the exposure to the parasites and the susceptibility to infection. There are references putting the weight of reason on one or another criterion or on a combination of both (Lester 2012; Tinsley et al. 2019; Warburton and Vonhof 2018). However, the variance of parasite distribution in hosts is mostly constrained by the value of the mean (Poulin 2013). Thus, according to the meta-analysis studies of Shaw and Dobson (1995) and Poulin (2013), it was observed that the regression between log of the mean and the log of the variance of the number of parasites per host, showed a r^2 of 0.87–0.88, which indicates that only 12% of the variability

in the distribution of parasites remains under the explanation of other factors. In the present study, the five trematode populations analyzed showed an r^2 of 0.87 (adjusted $r^2 = 0.83$) which is similar or slightly lower than the values established in the previous reviews. This reflects that, in the present study, it is the abundance of parasites (the mean) which determines the level of aggregation observed and 17% of the variability would remain dependent on other factors (Fig. 6).

The distribution of parasites and their corresponding infection parameters are not static but dynamic and can change temporally and spatially (Poulin 2007). In the present study, seasonality plays a role in the distribution of *A. burminis* in *H. fasciata* and *P. cupanus* and *C. complanatum* infection in *P. cupanus*. The individual level heterogeneity, both inside the host and in the host's environment, provides a mechanism behind patterns of parasite aggregation (Warburton and Vohnhof 2018). Precisely, environmental factors can influence the changes in abundance and, therefore, in the aggregation pattern. Parasite distribution patterns are shaped by a suite of abiotic and biotic factors, which directly and/or indirectly influence both parasites and their hosts (Ostfeld et al. 2005; Paterson et al. 2019).

In lotic freshwater ecosystems, the combined influence of the unidirectional water flow and the mobility of the most mobile hosts are considered to be primary drivers that structure the distribution of parasites, especially those with complex life cycles such as trematodes (Blasco-Costa et al. 2013; Salgado-Maldonado et al. 2014; Paterson et al. 2019). According to Price (1990) attention needs to be given to host properties such as geographic range, abundance and reproduction because these factors drive the nature of parasite assemblage. In the present study, the distribution of metacercariae was mainly influenced by the differences in ecological parameters prevailed in different host localities. This is true in the case of obligate, multi-host parasites, like trematodes as the successful completion of their life cycles depend on the presence and abundance of suitable hosts (Blakeslee et al. 2011). Trematodes are highly specific to snail hosts (typically infecting only one or few species), but infection to second-intermediate and definitive hosts is often more general (Graczyk et al. 1997); even then, specific groups of host species are typically targeted. Therefore, the availability of suitable hosts explain the presence or absence of specific parasite species in an area (Blakeslee et al. 2011).

The present study represents the first approximation to the infection patterns by metacercariae in *H. fasciata* and *P. cupanus*. The authors consider this information as essential, to be more precise, to properly direct to make efforts in the formulation of new working hypotheses in relation to infection by parasitic trematodes in the

freshwater fishes of the Western Ghats (India), an area of high ecological interest.

Author contributions All authors contributed to this study. P.J. J.: collection of fishes, isolation of parasites, concept of the manuscript and writing of the manuscript. P.A.: statistical analysis and writing of the manuscript. P.K.P.: concept of the manuscript and editing of the manuscript. All authors read, revised, and approved the final draft.

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Declarations

Conflict of interest The authors declare that there is no competing interest.

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