Intraguild interactions between five congeneric species of *Thaparocleidus* (Monogenoidea) from the freshwater shark *Wallago attu*, Lucknow, India

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**Abstract**

The fish host (*Wallago attu*) and monogenoid parasite (*Thaparocleidus* sp.) have been recorded during 2 years (2011 and 2012) to investigate intra/interspecific interactions among query species (*T. sudhakari* (Gusev, 1976) Lim, 1996; *T. indicus* (Kulkarni, 1969) Lim, 1996; *T. gomtius* (Jain, 1952) Lim, 1996; *T. yogendraii* Agrawal, 1981 and *T. wallagonius* Jain, 1952) in the light of parasitic worm burden, morphological and molecular data. Each species is highly host specific (oioxenous), with a specialized functional niche. Being niche specialists, they exhibit niche overlapping along with co-existence due to reproductive barrier. Furthermore, a molecular marker, affirming clear-cut genetic variation in spite of structural entities, provides evidence for infra-speciation as well as co-speciation.

**Introduction**

*Wallago attu* Bloch and Schneider, 1801, a freshwater, demersal fish, commonly known as ‘padhan’ in Indian fish markets, has been examined extensively in India and abroad for monogenoid infection (species of three genera – *Thaparocleidus*, *Bychowskyella* and *Mizelleus* – are confirmed so far). Of these, the genus *Thaparocleidus* is an iconic ecological model, having five oioxenous and co-existing species on the host, collected throughout the year from the River Gomti, district Lucknow. Customarily, monogenoideans form species complexes due to their large populations. Such complexes may arise through host–parasite co-evolution. Since parasites have opportunities for sympatric speciation, they exploit different microhabitats within the same host. Earlier studies conducted in India, on the genus *Thaparocleidus*, were either on taxonomical aspects or on phylogeny (Mollaret *et al.*, 2000; Justine *et al.*, 2002; Šimková *et al.*, 2006a, 2013; Pandey & Agrawal, 2008; Wu *et al.*, 2008; Rajvanshi & Agrawal, 2013, 2014a, b; Rajvanshi *et al.*, 2014; Tripathi *et al.*, 2014). Rajvanshi *et al.* (2014), while establishing the phylogenetic relationship among various species of the genus *Thaparocleidus*, using a molecular marker, validated these five species. Monogenoideans are particularly suitable parasites for revealing novel insights into host ecology and evolution (Xavie *et al.*, 2015). They are also economically important for cultured and feral fish because they cause diseases and foster secondary infections, more specifically in overcrowded culture systems (Cusack & Cone, 1985; Scholz, 1999; Jones, 2005; Ogawa, 2005), due to shorter generation period, direct life cycle and host specificity that intensify infection rate (Turgut & Akm, 2003). Thus, in the present study, component community, seasonal rhythmicity, intensity of infection, microhabitat preference, host specificity index, species co-existence, niche size, niche overlap, intra/interspecific aggregation and phylogeny have been examined within same microhabitat.

**Materials and methods**

*Collection of fish and examination of gills*

Three fish were collected monthly for *Thaparocleidus* sp., from the River Gomti, Lucknow, near the Mohan Meakin

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brewery (26°52'28"N, 80°55'18"E), at regular intervals from January 2011 to December 2012, with the help of fishermen using a floating gill net, trammel net and angling. Gills were excised from the fish into Petri dishes and subsequently examined for parasites under a phase-contrast microscope (Olympus BX 51, Tokyo, Japan). Collection and preservation of parasites was done using standard techniques (Agrawal et al., 2006). From 72 specimens of W. attu, 10,920 specimens of Thaparocleidus were collected. Of these, 3186 parasites were T. sudhakari (GenBank accession no. JX984666), 2865 T. indicus (JX960419), 2676 T. gomtius (KC962229), 2064 T. yogendraii (JX984665) and 129 T. wallagonius (JN020351). Calculation of component community was based on the 2-year dataset. A line-chart graph of infection intensity was plotted for each species. Microhabitat preference was quantified by counting the number of each species on gill arches. The following calculations were also included to analyse the dataset.

Host specificity and species aggregation

The host specificity index ($S_i$) of each species has been calculated according to Rohde’s first sub-section of his third law (Rohde, 1980a):

$$S_i = \frac{1}{P_i/n_j} = \frac{1}{n_j}$$

where

$$P_i = \frac{n_i}{n_j}$$

$n_i$ and $n_j$ are number of individual of $i$ and $j$ species.

Species co-existence ($A_{12}$) was calculated according to Ives (1991), Jeannik & James (1991) and Stevensler (1996). Intraspecific aggregation dominates over interspecific aggregation if the value of $A_{12}$ is greater than one.

$$A_{12} = \frac{(J_1 + 1)(J_2 + 1)}{(C_{12} + 1)^2}$$

Here $J_1$ and $J_2$ are intraspecific aggregation for the species one and two.

Intraspecific aggregation ($J_i$) was calculated according to Ives (1988, 1991) followed by Morand et al. (1999), where $J_i$ is the proportional increase in the number of conspecific competitors experienced by random individual relatives to a random distribution (Šimková et al., 2000):

$$J_i = \frac{\sum_{j=1}^{p} n_j(n_{ii}-1) - m_i}{m_i} = \frac{V_i - 1}{m_i}$$

Here $n_{ii}$ is a number of species 1 on gill arch $i$, $m_i$ is a mean number of species 1 per gill arch and $V_i$ is variance in number of species 1 on one host. $P$ is the number of gill arches. If the value of $J_i = 0$, individuals are randomly distributed. If $J_i = 0.5$, there is an increase of 50% in the average number of conspecifics that occur on the same patch above a random distribution (Šimková et al., 2000).

Interspecific aggregation ($C_{12}$) was calculated according to Ives (1988, 1991). Interspecific aggregation is a proportional increase in the number of hetero-specific competitors in relation to random association:

$$C_{12} = \frac{\sum_{j=1}^{p} \frac{n_i n_j - m_i m_j}{m_i m_j}}{m_i m_j} = \frac{Cov_{12}}{m_i m_j}$$

where $n_i$ and $n_j$ are the numbers of species 1 and 2 on gill arch $i$; $m_i$ and $m_j$ are the mean numbers of species 1 and 2 per gill arch, $P$ is the number of gill arches, $Cov_{12}$ is the covariance between a pair of species. Two species are positively correlated if $C_{12} > 0$ while negatively if the value is $C_{12} < 0$.

Niche size and overlap

Levin’s niche breadth ($B$) was calculated according to Colwell & Futuyma (1971), where $P_i$ is the proportion of individuals of a species found on one gill arch:

$$B = \frac{1}{\sum_{j=1}^{p} \left(\frac{p_j}{P_i}\right)^2}$$

Niche overlap was calculated using Renkonen’s index (Geets et al., 1997), where $P_{ij}$ is the proportion of individuals of species $i$ on gill 1 and $P_{ji}$ is the proportion of individuals of species $j$ on gill 1:

$$1 - \frac{\sum |P_{ij} - P_{ji}|}{2}$$

Phylogeny

Sequences retrieved from GenBank (National Center for Biotechnology Information) were analysed by MEGA software, version 6.06 (Tamura et al., 2013) for reconstruction of the phylogenetic tree (without outgroup). Minimum-evolution (ME), neighbor-joining (NJ) and unweighted pair group method with arithmetic mean (UPGMA) were utilized to test genetic diversity significantly affecting morphological variations and niche overlapping among species.

Results

Component parasite community and seasonality

All five Thaparocleidus sp. co-exist splendidly on the same host, affecting each other to a lesser extent. During this study, a total of 10,920 worms were observed with an infection intensity of 151.66 parasites/fish examined, none from fries/fingerlings of W. attu. Thaparocleidus sudhakari was the most abundant, with 29.17% intensity, followed by T. indicus (26.23%), T. gomtius (24.50%), T. yogendraii (18.90%) and T. wallagonius (1.18%). Thaparocleidus wallagonius (48.61 ± 32.57 prevalence which is 100 for rest four species) represents the apex of the community, followed by T. yogendraii, T. gomtius, T. indicus and T. sudhakari (having the maximum number within the community). All five Thaparocleidus sp. are oioxenous, sympatric (Lerssuthichawal & Lim, 2005; Rajvanshi et al., 2014) and structurally distinct (fig. 1).

Each species had a characteristic pattern (more or less similar in both years) of seasonal rhythmicity (winter/summer/monsoon peak) that never disturbs each other even in high infection phases. Thaparocleidus wallagonius...
Fig. 1. Morphological variations in male reproductive parts of the five *Thaparocleidus* sp.
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Fig. 2. The intensity of infection of (A) Thaparocleidus wallagonius (– – – –), T. yogendraii (— — —) and T. gomtius (•••••) (B) T. indicus (— — —) and T. sudhakari (...) in the freshwater shark Wallago attu in the River Gomati from January 2011 to December 2012.

(being the type species) was found throughout the year, at very low intensity (fig. 2A); T. yogendraii prevailed during winter (February) and autumn (October) (fig. 2A), T. gomtius also showed two peaks, spring (April) and autumn (October). Thaparocleidus sudhakari showed two peaks, winter (mid-December and January) and summer (June), and T. indicus showed three peaks, winter (February), spring (April) and autumn (mid-September and October), the second peak being prominent. The first peak showed the time of initial recruitment, followed by a second peak due to increase in intensity of infection (fig. 2B). Thaparocleidus gomtius and T. indicus showed similar peak values in the spring (April). The peak values of T. sudhakari were higher than those of the other species throughout year (fig. 2A and B).

Microhabitat preference

All four pairs of gill arches were infected with the five congeners of Thaparocleidus. Thaparocleidus wallagoniius expressed more-or-less similar preference for all the four pairs of gill arches. However, T. yogendraii, T. indicus, T. gomtius and T. sudhakari showed a preference for the first and fourth gill arches. Thaparocleidus sudhakari prevailed over the rest of the species (with respect to prevalence and intensity of infection). The supremacy of T. sudhakari on gill arches was followed by T. gomtius, T. yogendraii and T. wallagoniuns. Thaparocleidus sp. demonstrated a proportionate relationship between infection intensity and host size.

Host specificity and species aggregation

The host specificity (HS) index was 1 for all congeners of Thaparocleidus under study. This index approaches 1 for parasites restricted to a single host species (Rohde, 1980a). Thus, all these five Thaparocleidus sp. were strictly host specific for W. attu, regardless of the fact that this is a generalist genus. The HS index of each Thaparocleidus sp. remained same throughout the year in both sexes and different climatic conditions.

These five Thaparocleidus sp. co-exist. The value of species co-existence (A_{12}) was >1 for all species, proving intraspecific aggregation over interspecific aggregation (table 1). Species co-existence ranged between 6.087 and 16.399. The highest A_{12} value was assigned for the pair T. gomtius–T. indicus (16.399), similar to those for the pairs T. sudhakari–T. indicus (16.334) and T. sudhakari–T. gomtius (16.143).

Each Thaparocleidus sp. had an intraspecific aggregation value >0, confirming intraspecific aggregation of Thaparocleidus sp. within the same habitat. The values of intraspecific aggregation for T. gomtius, T. indicus and T. sudhakari were more or less similar, followed by those for T. yogendraii and T. wallagonius. Thaparocleidus wallagonius had the lowest value for intraspecific aggregation (table 2). The infection intensity and intraspecific aggregation expressed a positive correlation for all species, with significant values for T. yogendraii and T. gomtius (table 3). Intraspecific aggregation and niche size revealed negative interactions for all species, except T. wallagonius (table 3).

The interspecific aggregation (C_{12}) for ten pairs of Thaparocleidus sp. was >0, exhibiting positive values (table 1). The highest value of interspecific aggregation was assigned for T. yogendraii–T. gomtius and least for the pair T. wallagonius–T. indicus. The high value of C_{12}, indicating enhanced co-existence by reduction of interspecific competition due morphological variation, seems plausible reason that allows species to co-exist (Šimková et al., 2000, 2002).

Niche size and niche overlap

The niche size ranged between 3.44 and 3.78 for all species (table 2). Each species had its own separate niche albeit of different population dynamics along with slight niche overlap, spanning between 0.764 and 0.893 (table 1). Maximum niche overlap was between T. gomtius and T. yogendraii (0.893) and least was between T. wallagonius and T. yogendraii (0.764). Nevertheless, parasites having different reproductive parts occupy the same niche. Surprisingly, species having more-or-less similar sclerotized parts may also have niche overlap if there is less interspecific competition.
Table 1. Correlation values for species of Thaparocleidus relative to co-existence ($A_{12}$) / interspecific aggregation $C_{12}$ ($\times 10^{-2}$)/niche overlap.

<table>
<thead>
<tr>
<th>Species</th>
<th>$A_{12}$</th>
<th>$C_{12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. wallagonius</td>
<td>6.087/0.998/0.764</td>
<td>6.269/1.008/0.766</td>
</tr>
<tr>
<td>T. yogendraii</td>
<td>–</td>
<td>15.783/1.680/0.893</td>
</tr>
<tr>
<td>T. indicus</td>
<td>–</td>
<td>16.399/1.121/0.879</td>
</tr>
<tr>
<td>T. gomtius</td>
<td>–</td>
<td>16.344/0.951/0.880</td>
</tr>
<tr>
<td>T. sudhakari</td>
<td>–</td>
<td>16.143/1.670/0.878</td>
</tr>
</tbody>
</table>

Table 2. Mean values ($\pm$ SD) of abundance, intensity, intra-specific aggregation ($J_1$) and niche size in five species of Thaparocleidus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance ($\pm$ SD)</th>
<th>Intensity ($\pm$ SD)</th>
<th>$J_1$ ($\pm$ SD)</th>
<th>Niche size ($\pm$ SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. wallagonius</td>
<td>1.79 ± 1.56</td>
<td>3.45 ± 0.87</td>
<td>0.60 ± 1.27</td>
<td>3.44 ± 0.35</td>
</tr>
<tr>
<td>T. yogendraii</td>
<td>28.67 ± 17.58</td>
<td>28.67 ± 17.58</td>
<td>2.98 ± 0.22</td>
<td>3.78 ± 0.12</td>
</tr>
<tr>
<td>T. indicus</td>
<td>39.79 ± 24.87</td>
<td>39.79 ± 24.87</td>
<td>3.09 ± 0.17</td>
<td>3.76 ± 0.15</td>
</tr>
<tr>
<td>T. gomtius</td>
<td>37.17 ± 26.57</td>
<td>37.17 ± 26.57</td>
<td>3.10 ± 0.28</td>
<td>3.71 ± 0.19</td>
</tr>
<tr>
<td>T. sudhakari</td>
<td>44.25 ± 32.16</td>
<td>44.25 ± 32.16</td>
<td>3.07 ± 0.20</td>
<td>3.77 ± 0.18</td>
</tr>
</tbody>
</table>

Table 3. Intensity of infection ($\pm$ SE) levels in five species of Thaparocleidus, calculated as $\log(x + 1)$, versus intraspecific aggregation as $\log(x + 2)/$interspecific aggregation as $\log(x + 2)$ versus niche size as $\log(x + 1)$, corrected for total intensity of infection as $\log(x + 1)$.

<table>
<thead>
<tr>
<th>Species</th>
<th>$R^2$</th>
<th>$b$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. wallagonius</td>
<td>0.039</td>
<td>0.208/0.182</td>
<td>0.355/0.381</td>
</tr>
<tr>
<td>T. yogendraii</td>
<td>0.468</td>
<td>9.205/–1.210</td>
<td>&lt;0.001/&lt;0.001</td>
</tr>
<tr>
<td>T. indicus</td>
<td>0.056</td>
<td>3.802/–0.920</td>
<td>0.265/&lt;0.001</td>
</tr>
<tr>
<td>T. gomtius</td>
<td>0.191</td>
<td>4.952/–1.169</td>
<td>0.033/&lt;0.001</td>
</tr>
<tr>
<td>T. sudhakari</td>
<td>0.005</td>
<td>1.113/–0.863</td>
<td>0.750/&lt;0.001</td>
</tr>
</tbody>
</table>

Phylogeny

All three methods of phylogeny (without an out-group) performed for the queried species of the genus Thaparocleidus depicted similar results (fig. 3). The aligned dataset consisted of 363 parsimony-informative sites and three conserved sites. All species are descended from a common ancestor via intra-host speciation (within a host). In spite of sharing an ancestral node, they are genetically different. Highest negative free energy, topology and pattern of the loops within secondary structures of RNA also support genetic discrimination of the species under study (Rajvanshi et al., 2014).

Discussion

All the five species of the genus Thaparocleidus are specialized to a specific niche and exhibit overlapping to some extent, due to co-existence. Rohde (1980b) has also supported overlapping for monogenoidean multispecies communities. Nevertheless, parasite abundance does not affect survival rate or species diversity (Rohde, 1980b, 1981). Random distribution and host size (which provides a greater microhabitat) to flourish, with less intra/ interspecific competition) may dwindle the expected number of congeneric species (Pianka, 1983).

These monogenoideans demonstrate biological rhythmicity (fig. 2A and B), a peculiar and indispensable feature for maintenance of population size and interspecific competition among allied and non-allied parasite species within the same microhabitat (Maclung, 2006; Ray & Agrawal, 2014). High intensities of parasites support expansion of the microhabitat and intra/ interspecific competition (Rohde, 1991). Relative performance (throughout the year) of each species is mainly under density-dependent regulation and environmental stochasticity (Wilson & Yoshimura, 1994; Šimková et al., 2001a). Tripathi et al. (2010) have reported similar observations for T. gomtius, T. wallagonius and M. indicus on W. attu.

Monogenoidean parasites also display microhabitat preference (Cerfontaine, 1896, 1898). All Thaparocleidus sp. appear well adapted to suitable and specific microhabitats, indistinguishably infecting right and left gill arches. The preference for gill arch (numbers 1–4 of each side) is influenced by aquatic pressure within the habitat (Wootten, 1974; Kadlec et al., 2003). Thaparocleidus gomtius, T. indicus, T. sudhakari and T. yogendraii had less affinity for the second and third gill arches (despite the gill area decreasing from first to fourth arch) (Gutierrez & Martorelli, 1999) as they receive strong water pressure in comparison to the first and fourth arches. However, T. wallagonius showed similar preference for all arches throughout the year. This may be due to lower infection compared to that of the other species. Mature parasites prefer secondary gill lamellae, especially terminal edges, as axial filaments are physiologically dead (Hughes, 1966; Turget et al., 2006), and immature parasites ignore the extremities, to minimize the chance of being washed
Thaparocleidus sp. are reproductively as well as genetically differentiated, morphologically more-or-less similar and promote communication (Ramasamy et al., 1985; Rodhe, 1991), ascertaining survival of their progeny.

Monogenoideans are known to be highly host specific (Rohde, 1994; Rajvanshi et al., 2015) and may evolve from generalist parasites (Kawecki, 1998). These parasites can be used as biological tag (Rajvanshi et al., 2015; Agrawal et al., 2016). They co-speciate more closely along with their hosts than generalist parasites (Humphery-Smith, 1989; Sasal et al., 1998), show stark variations (Sasal et al., 1998; Bush et al., 2001; Kaci-Chaouch et al., 2008) and develop structural specificity due to selection pressure within the microhabitat (Kaci-Chaouch et al., 2008). Host-specific parasites exhibit less variation in sclerotized parts than generalists (Šimková et al., 2001b; Jarkovsky et al., 2004). Ecological availability of the host throughout the year, or not getting the chance to switch hosts, could be possible grounds for host specificity (Šimková et al., 2006b).

Lower interspecific competition among congeners could be a cause of co-existence and species diversity. Species having different sets of characteristics (copulatory organ and haptoral armature) co-exist in the same microhabitat due to spatial segregation (Rohde & Hobbs, 1986; Šimková et al., 2002). Monogenoideans show a different niche size in species-rich habitats (Rohde, 1981). Space is the critical factor for niche segregation regardless of food resources, which are unlimited while the host survives (Rohde, 1991). Reproductive barriers to prevent species hybridization play a crucial role in niche segregation, rather than interspecific competition (Rohde, 1991; Šimková et al., 2002). However, the co-existence of species increases as the morphological variability and niche centre increases (Šimková et al., 2002). Apart from behavioural differentiation, morphologically more-or-less similar Thaparocleidus sp. are reproductively as well as genetically isolated and share an ancestral genome, pointing towards evolution from the same gene pool. The host–parasite phylogenetic congruity prediction is not possible here due to intra-host speciation (Bochkov & Mironov, 2008). The parasite–host association evolved contemporaneously but congeners of the parasite evolved sympatrically. Microhabitat specialization may be a cause of genetic divergence among these congeneric species (Eichler, 1966; Bochkov & Mironov, 2008). Gene duplication is the explanation for sympatric speciation/intra-host speciation (Huyse et al., 2005). Faster diversification of the parasite is responsible for multispecies complex formations within the same microhabitat, due to higher evolutionary rates in comparison to that of host species (Rohde, 1980b). Thus, Thaparocleidus demonstrates itself to be an iconic ecological model, paving the way for further investigations in other oioxenous species. The morphological and molecular data suggest that all five congeners live together harmoniously, employing seasonal variation in intensity of infection, reproductive isolation and genetic isolation as increasingly effective barriers to competition.

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### Conflict of interest

None.

### References


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