

Note on speciation of *Thaparocleidus*, Jain, 1952.

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Abstract.

Parasite duplication (i.e., intrahost speciation) was recognized as the most common event in *Thaparocleidus*, while the numbers of cospeciation and host switches were lower and similar to each other. When collapsing nodes correspond to duplication cases, our results suggest host switches in the *Thaparocleidus*-Siluridae system precluding congruence between host and parasite trees. We found that the morphometric variability of the parasite attachment organ is not linked to phylogeny, suggesting that the attachment organ is under adaptive constraint, Haptor morphometry is linked to host specificity, whereby nonspecific parasites display higher morphometric variability than specialists.

Key words: Monogenoidea, India, Speciation.

Material and methods:

Literature was collected from various sources and studies were done. Fishes were collected from the River Gomti at Lucknow, State of Uttar Pradesh, India (26° 45' -27°N; 80° 50' -81° 5'E) for investigation. Monogenoidean parasites found on them were counted. The gills were removed and preserved in 3% formalin diluted with lukewarm water. The methods of staining, mounting, and illustrating the dactylogyrids were those described by Kritsky, Thatcher & Boeger (1986). Measurements, all in μm , were taken using a calibrated micrometer; means are followed by the range and the number (n) of specimens measured in parentheses

1. Introduction

The speciation of free-living organisms is thought to be caused by two main mechanisms: allopatric speciation, which results from reproductive isolation due to extrinsic factors such as geographical barriers and nonallopatric speciation such as sympatric speciation, which requires intrinsic barriers for reproductive isolation. In parasites, speciation is usually linked to the evolutionary history of their host species, with host speciation inducing parasite speciation when each incipient host species has inherited parasite populations that subsequently diverge from a common ancestor. Therefore, the allopatric speciation of parasites may occur when extrinsic barriers prevent parasite reproduction among isolated host populations. For example, this can occur when the host species are geographically isolated. On the other hand, sympatric speciation (geographic sympatry, within host sympatry or within microhabitat sympatry) can occur when the isolation of parasite populations is maintained by intrinsic barriers and is therefore independent of host speciation events. Sympatric speciation could explain a large part of parasite diversity. According to Kunz, sympatric speciation is more likely to occur in parasites than in free-living organisms, considering that the isolation of parasite populations seems to be accomplished more easily than in free-living organisms. The isolation processes and intrinsic barriers among parasite populations, such as host choice when the parasite shows a local host preference or mate choice when mating between two parasites is impossible, most likely will lead to parasite sympatric speciation. Parasite sympatric speciation may occur within a single host species; that is, a parasite lineage has evolved within a single host species without any isolation of host populations. The key assumption here is that congeneric parasite species on the same host are sister species and that their occurrence is the result of one or more events of intrahost speciation

Cophylogenetic studies comparing the evolutionary histories of parasites and their associated hosts may help us to further explore parasite speciation mechanisms. Congruence of host and parasite phylogenies is considered evidence of cospeciation that is, the concurrent speciation of both associated

partners. However, congruent trees are not always linked to cospeciation. Incongruent phylogenies are often explained by host-switching events or parasite duplication. However, testing for cospeciation or codivergence (i.e., simultaneous speciation or divergence of host and parasite lineages, while cospeciation is a special kind of codivergence in which the end products of the divergence process are considered separate species) requires the combination of distance-based, tree-based, and data-based (these methods are used to determine the cause of topological incongruence between host and parasite trees) cophylogenetic methods. Indeed, discriminating between trees that are concordant as a result of codivergence and trees that are concordant for reasons unrelated to codivergence necessitates the comparison of topological similarities between not only host and parasite trees but also timing of events. Speciation in parasites has been mostly explained by their life-history traits, such as host specificity. Generally, a parasite living on/in one host species is considered a specialist, and a parasite living on/in at least two species is considered a generalist. Brooks and McLennan, hypothesized that the chance of colonizing new host species, that is, host switching, and the subsequent speciation are inversely related to the degree of host specificity, which supposes that cospeciation and intrahost speciation are more frequent in parasites having a narrow host range. For example, in the highly host specific chewing lice parasitizing pocket gophers, cospeciation was found to be the main speciation event .

Monogenoideans, a group of mostly ectoparasitic flatworms predominantly found in fishes, seem to be an ideal model for investigating parasite diversification for at least three reasons. First, monogenoideans are a highly diverse parasite group in terms of species richness . Second, many monogenoidean species tend to be host specific, that is, infecting only one or a few host species and also niche specific, that is, restricted to a particular habitat within the host species . Third, monogenoideans are parasites with a direct life cycle (only one host species is involved in their life cycle), which may simplify the analyses of host-parasite associations compared to endoparasites with a complex life cycle (including intermediate and definitive host species throughout various stages of the life cycle). To date, several studies have investigated the speciation and diversification of different congeneric monogenoidean species. These studies do not show strong patterns of cospeciation, despite

the high host specificity of monogonoidean parasites, but they suggest that monogeneans mostly diversify either through host switching or by intrahost speciation. Host specificity, varying between the different monogonoidean models investigated, is considered to be an important parasite trait involved in monogonoidean speciation processes.

Monogonoideans possess a posterior attachment organ, called a haptor, which is supposed to be linked to both specialization and adaptation. Morand et al. hypothesized that a link between morphological and phylogenetic distances may reflect a nonadaptive trend due to a high phylogenetic inertia, with sister species possessing similar haptors because they have inherited them from a common ancestor. Conversely, a link between the morphometrics of the monogonoidean haptor and host specificity may reveal a potential adaptation. Indeed, a higher variability of the attachment organ was shown in generalists compared to specialists in two groups of monogonoidean species.

Discussion:

Thaparocleidus Species: Morphological versus Molecular Species Concept

In monogonoideans, species identification is generally based on the morphology of two sclerotized organs, the attachment organ (the haptor) and the reproductive organ, including the copulatory piece and the vagina. The morphology of the haptor is considered useful for parasite determination at the genus level, while the reproductive organ is more suitable for identification at the species level, probably because of its higher rate of change. However, in some cases, identification is problematic at the generic as well as the specific level. Therefore, molecular identification is a helpful tool in resolving taxonomic problems in cases where the morphological “boundaries” among monogonoidean genera or species groups are ambiguous.

Thaparocleidus parasitizing Siluridae and *Thaparocleidus* parasitizing Siluridae form two divergent genetic lineages. The morphology of the male copulatory organ of *Thaparocleidus* was suggested as a key determinant for separating the clades recovered in molecular phylogenetic reconstruction

Moreover, the evolution of sclerotized organs is not neutral and seems to be under adaptive constraints in case of the attachment organ. In our study, the morphological variability of the haptor was not linked to phylogenetic distances, suggesting that the morphological variability of these sclerotized organs is not inherited from a common ancestor and may be under adaptive constraint.

4. Morphometric and Molecular Variability versus Host Specificity

Interspecific variability in ITS1 sequences and in haptor morphometry was previously shown in generalist monogenoideans such as species belonging to *Dactylogyrus* and *Lamellodiscus*. Moreover, *Lamellodiscus* generalists have a higher intraspecies molecular variability and a higher variance of haptor morphometry than do specialists. In our study, we also found that the variance in haptor morphometry is higher in *Thaparocleidus* generalists than in specialists. Kaci-Chaouch et al. proposed two alternative hypotheses to explain why the variance in haptor morphometry is higher in generalists than in specialists. Generalists exhibit a higher variance because (i) they use different host species representing a wide range of niches, which can exert different pressures on morphology, or (ii) they have a higher morphometric variability of the attachment organ which may allow parasites to colonize more host species. Therefore, morphometric variability can have a major impact on parasite speciation processes regardless of host speciation by restricting specialists within a particular host and habitat, thereby giving generalists the capability to have a larger host range and/or colonize several habitats.

Speciation and Diversification in *Thaparocleidus*

The concept of sympatric speciation as an evolutionary diversification process remains controversial. According to Coyne, there are four main requirements needed to prove sympatric speciation. The first is that the species must be largely or completely sympatric. In our case, if we consider the host species as a unit, all parasite species found within the same given host species are considered to be sympatric species. Secondly, these sympatric species must show reproductive isolation. It was demonstrated in

other monogenoidean groups that congeneric species found in the same host species and occupying adjacent niches within the host (i.e., gill parts) differ in the morphology of their copulatory organs. Thirdly, the sympatric species must be sister species, which is shown in our phylogenetic analyses where most *Thaparocleidus* species from a single host species form a monophyletic group. Fourthly and finally, the species did not seem to have undergone an allopatric diversification phase. However, a more intensive survey and analysis of the *Thaparocleidus* species will be needed to justify this assumption.

Sympatric speciation can usually be encountered when closely related species live in isolated island-like habitats. Host species are considered as islands for parasites, and in view of the parasite life cycle we can expect parasite speciation to occur at a higher rate than host speciation. This faster pace of evolution also favours intrahost speciation. Sympatric speciation in monogenoideans has previously been observed in *Dactylogyrus* species parasitizing cyprinid fish in Central Europe. In this system, the authors suggested that parasite diversification can be explained by sympatric speciation events (i.e., intrahost speciation). Intrahost or sympatric speciation is linked to reproductive isolation of sympatric parasite populations. Different mechanisms have been proposed to explain the reproductive isolation of parasites such as habitat selection (preferred niches are its consequence) or mate choice. On the basis of tree-based cophylogenetic analysis using different event cost schemes, sympatric speciation (i.e., intrahost speciation) also appears as the dominant coevolutionary event involved in *Thaparocleidus* diversification. However, our study also evidenced some host switches in the *Thaparocleidus*-Siluridae system. Giraud et al. showed that certain pathogen life traits (i.e., production of numerous propagules, gene exchange occurring within hosts, linkage of traits experiencing selection, and strong selection imposed by the hosts) likely render them prone to rapid ecological speciation by host shifts (i.e., speciation by specialization onto a novel host). As many such life traits have been recognized also for monogenoidean fish parasites, this may explain the evidence of host switches documented by cophylogenetic analyses in monogenoidean parasites.

to topology The overall congruence between the *Thaparocleidus* and Siluridae phylogenies was statistically significant according -based and distance-based methods. Using a tree-based method, a nonsignificant global fit between the phylogenies of *Thaparocleidus* parasites and silurid hosts was found only using the model with a higher cost for duplication than for host switch. When considering

the fact that duplication is the most numerous coevolutionary event in congeneric monogenoideans parasitizing freshwater fish hosts, duplication is probably not so costly as host switch is (because many monogenoidean species are host specific; thus, they have a limited ability for dispersal to other host species). Therefore, the model with the cost of 2 for duplication and 1 for host switch seems to be less realistic.

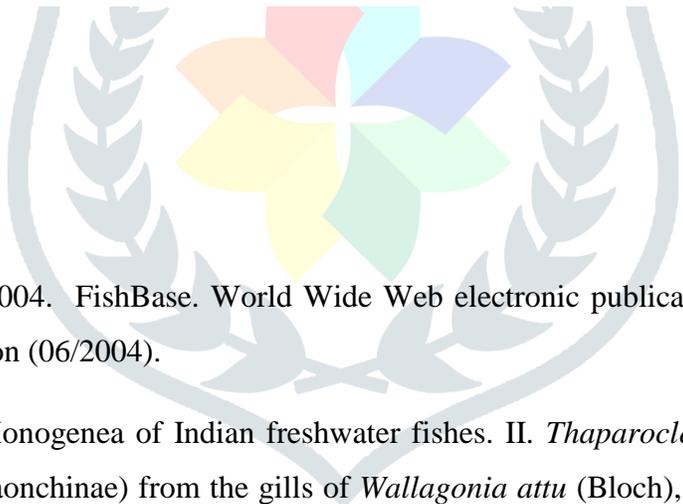
. Huyse and Volckaert on the basis of tree-based methods found an overall fit between the phylogenies of *Gyrodactylus* parasites (viviparous monogenoideans) and goby hosts, but an absolute timing of speciation events in hosts and parasites ruled out the possibility of synchronous speciation. Thus, they proposed that phylogenetically conserved host switching mimics the phylogenetic signature of cospeciation.

Bentz et al. studied the evolution of African Polystoma, endoparasitic monogenoideans of neobatrachian hosts, and proposed that distinctive larval behaviour of polystomes engenders isolation between parasite populations which precludes sympatric speciations, and thus cospeciation is another factor of diversification of Polystoma in the African continent. However, the majority of previous cophylogenetic studies on congeneric monogenoideans parasitizing fish did not report cospeciation. De Vienne et al. in their review study showed that convincing cases of cospeciation in host-parasite and host-mutualist associations are very rare and host switches may be the dominant mode of speciation over cospeciation. In addition, they suggested that cophylogenetic methods overestimate the occurrence of cospeciation. Different processes may generate apparent cospeciation. Our study indicates that such apparent cospeciation in *Thaparocleidus*-Siluriformes may be generated by intrahost duplications and/or also caused by host-switching events. The sympatric occurrence of some silurid species may more likely support the evidence of host switches than cospeciation in *Thaparocleidus* diversification; for instance, Siluridae. live in the same basin, which could facilitate host switching.

Discussion

Our study of closely related parasites within a relatively small geographical area emphasizes particularly that intrahost speciation is the dominant coevolutionary event in *Thaparocleidus* species diversification favored by high specificity. Our study may indicate that host switches rather than cospeciation play a more substantial role in *Thaparocleidus* diversification. However, Siluridae speciation is closely related to tectonic events and the variation of sea levels we then expected a similar pattern in parasite evolution. Therefore, to infer a formal conclusion on the role of cospeciation and host switching for *Thaparocleidus* diversification, we need to study these monogenean species on a broader geographical scale also including additional host species. Our study indicates that the morphological variability of attachment organ in *Thaparocleidus* parasites is not inherited from a common ancestor and could be potentially under adaptive constraint.

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