Trypanorhynch Assemblages Indicate Ecological and Phylogenetical Attributes of Their Elasmobranch Final Hosts

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Abstract: This study explores non-metric multidimensional scaling (nMDS) as a tool for investigating parasites as indicators of the elasmobranch biology. An attractive feature of nMDS is its ability to allow assemblage-level parasite data to be simultaneously applied to questions of host biology. This method was examined using the tapeworm order Trypanorhyncha Diesing, 1863, which is known to be transmitted among their hosts through the marine food web (via predation), can unambiguously be identified in the intermediate and final hosts, and has the potential as an indicator of the host feeding biology. Our analyses focused on trypanorhynch assemblages in elasmobranchs as definitive hosts. The relationships between trypanorhynch assemblages and the depth, feeding ecology, habitat, and phylogeny for all sharks were complex, but we found that depth distribution, diet composition and habitat type were the major influencing factors. Several species of sharks showed different characters than known from their descriptions that could be attributed to the change of shark behavior or the trypanorhynch host path. The relationship between the trypanorhynch assemblage and factors for carcharhiniform species alone was more robust than for all sharks. In the carcharhiniform analysis, the relationship between habitat type and trypanorhynch assemblage was most remarkable. Overlapping host ecology was evident even in phylogenetically-distant related hosts.

Keywords: Biological indicator; cestodes; classification; elasmobranchs; host specificity; host switch; phylogeny; feeding ecology; Trypanorhyncha

1. Introduction

Elasmobranchs are difficult to observe in nature and information on rare or less-frequently caught species is scarce. This especially concerns aspects of their ecology, main habitat, depth range, and most important prey items. Their prey is rapidly digested and affiliation, e.g., to mainly fish or invertebrate-feeding activities, is difficult. Fish parasites have been widely used as biological indicators for the host ecology, including their zoogeographical distribution, migration, and feeding behavior [1]. However, the methods applied for the elasmobranchs are, so far, limited due to often restricted and unpredictable catches and less availability of specimens to study.

The cestode order Trypanorhyncha Diesing, 1863, is considered to be a basal tapeworm group of elasmobranchs. Its members can be characterized by a scolex with a tentacular apparatus that can

be used for classification [2–5]. The most recent revision of the trypanorhynchs subdivided the order into five different superfamilies and 15 families [6]. The first large-scale molecular phylogenetic analyses of this group included trypanorhynch species belonging to all superfamilies and 12 of the 15 recognized families [7,8]. The analysis of complete simple sequence repeats DNA (ssrDNA) and partial long sequence repeat DNA (lsrDNA) (D1–D3) revealed high resolution and the presence of a basal split, representing the shark- and ray-inhabiting lineages as new suborders. These results gave, in part, support for the trypanorhynch classification by Palm [6] as well as more insight into the intrafamily phylogenetic relationships within the Trypanorhyncha. The host-parasite-checklist of these cestodes indicated that some of the superfamilies and families seem to have host preferences [6], while the patterns of others can be explained by host switches, even between rajiform and selachiform elasmobranchs [7].

The ~300 described species of the order Trypanorhyncha so far exhibit the following general life-cycle pattern [6,9–11]. The first intermediate host becomes infected when it consumes an oncosphere or free-swimming coracidium larva. The first parasitic larval stage is a procercoid; the limited available data suggest that this stage parasitizes copepods. Second intermediate hosts include a wide array of marine animals, but invertebrates and teleosts figure particularly prominent at this level. Unlike most groups of tapeworms, the trypanorhynch final larval stage, though having a different morphology (plerocercoid, plerocercus, merocercoid), is generally identifiable to species because it exhibits the adult hook pattern, a pattern on which much of the taxonomy of the group is based [4–6]. Some trypanorhynchs use a paratenic host following the final intermediate host; this host serves to bridge food-web gaps. As adults, trypanorhynchs parasitize the spiral intestine of sharks and rays (elasmobranchs). Fortunately, it is common for individual elasmobranch species to host multiple trypanorhynch species and, thus, assemblage-level data are readily available for many elasmobranchs that serve as definitive hosts.

Our study uses non-metric multidimensional scaling (nMDS) as a tool for investigating parasites as indicators for selected ecological attributes and the phylogeny of their elasmobranch final hosts. A particularly novel and attractive feature of nMDS is the possibility to allow assemblage-level parasite data to be simultaneously applied to questions of host biology. The method was examined using trypanorhynch tapeworm assemblages, which have obvious potential as indicators of host feeding biology in the elasmobranch. The complex life-cycles of these parasites, each of which involves a minimum of three different hosts and three distinct life-cycle stages that vary in their degree of host specificity [11] also suggest trypanorhynchs as appropriate indicators of other aspects of their host biology.

Among elasmobranchs, sharks (Selachii) were specifically chosen because they vary substantially in diet and habitat, which allows a wide spectrum of aspects of host biology to be explored. Within the Selachii, more detailed analyses were conducted on sharks of the order Carcharhiniformes (ground sharks). Comprehensive trypanorhynch data were available for this shark order, and provided a unique opportunity to perform nMDS analyses on a more precise taxonomic level. Factors concerning the shark biology included (1) taxonomy (order for the Selachii analyses, or family for the Carcharhiniform analyses); (2) habitat (benthic vs. pelagic); (3) depth distribution (above or below 200 m depth); and (4) diet (primarily invertebrates vs. primarily teleosts). Trypanorhynch assemblage dependence on these four factors was assessed by superimposing the different variables into the two-dimensional ordination plots resulting from nMDS analysis of the hierarchical agglomerative classification of shark species generated from their trypanorhynch assemblages. Trypanorhynch assemblages were considered to be appropriate as indicators of the biological factors that were found to be significantly correlated with the agglomerative classification.

2. Results

2.1. All Sharks

The result of the hierarchical, agglomerative classification (CLUSTER) of trypanorhynch assemblages of 50 species of Selachii revealed five major clusters of shark species at an arbitrary dissimilarity level of 78% (Figure 1). The two-dimensional ordination plots resulting from nMDS of this agglomerative classification, with sharks coded for taxonomic order, diet, habitat, and depth distribution are shown in Figure 2. The analysis of similarities (ANOSIM) revealed significant differences in trypanorhynch assemblage composition between sharks that primarily consume invertebrates versus those that primarily consume teleosts (global $R = 0.279$; $p < 0.01$), between those from benthic and pelagic habitats (global $R = 0.275$; $p < 0.01$), and between sharks occurring in deep versus shallow water (global $R = 0.327$; $p < 0.01$), thus supporting the significance of the major clusters resulting from the classification and ordination methods. With respect to taxonomy, the ANOSIM showed significant differences among shark orders (global $R = 0.381$; $p < 0.01$), suggesting a relationship between trypanorhynch genera and shark orders. In ANOSIM pairwise comparison, the trypanorhych assemblages of the Carcharhiniformes were significantly different from those of the Squaliformes (global $R = 0.546$; $p < 0.01$), the Orectolobiformes (global $R = 0.466$; $p < 0.1$), the Lamniformes (global $R = 0.312$; $p < 0.01$), and the Hexanchiformes (global $R = 0.275$; $p < 0.05$). In addition, the trypanorhynch assemblages of the Lamniformes were significantly different from those of the Orectolobiformes (global $R = 0.432$; $p < 0.01$) and Squaliformes (global $R = 0.355$; $p < 0.05$), and the assemblages of the Orectolobiforms were significantly different from those of the Squaliformes (global $R = 0.620$; $p < 0.01$). No significant differences were found between the trypanorhynch assemblages of any of the other 16 pairs of shark orders.
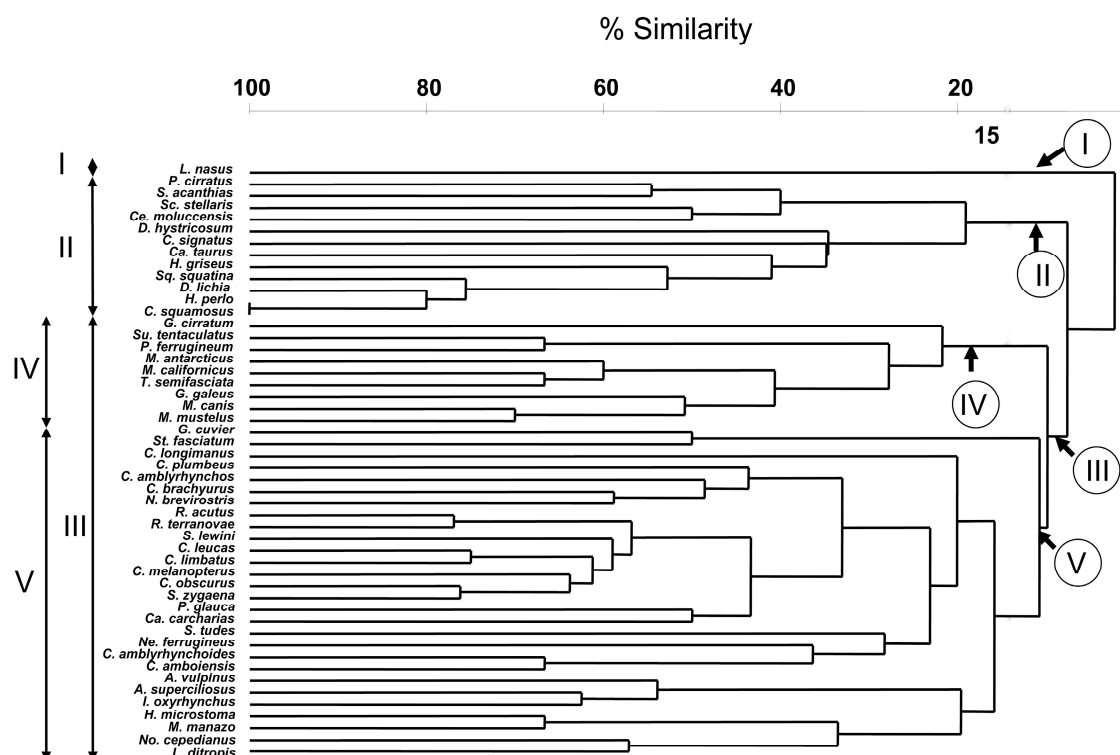

Figure 1. Cluster analysis of all sharks according to Trypanorhyncha assemblages. Roman numerals I–V indicate the five major clusters.

The following similarity percentage (SIMPER) analysis indicated which trypanorhynch genera contributed most to the differences between the studied shark species (Table S2). The average similarity was 22.29% for the pelagic sharks, with the genera *Nybelinia* Poche, 1926, *Otobothrium*

Linton, 1891, and *Dasyrhynchus* Pintner, 1928, accounting for a cumulative contribution of 54.30%. Including *Heteronybelinia* Palm, 1999, *Callitetrarhynchus* Pintner, 1931, *Tentacularia* Bosc, 1797, *Grillotia* Guiart, 1927, and *Floriceps* Cuvier 1817, the trypanorhynch genera reached a cumulative contribution of 91.51%. The benthic sharks which had an average similarity of 15.64% were characterised by five genera, *Dollfusiella* Campbell and Beveridge, 1994, *Nybelinia*, *Grillotia*, *Lacistorhynchus* Pintner, 1913, and *Gilquinia* Guiart 1927, which accounted for a cumulative contribution of 92.84%. The genera *Nybelinia* (12.48%), *Otobothrium* (8.26%), *Dasyrhynchus* (6.85%), *Heteronybelinia* (6.44%), and *Callitetrarhynchus* (5.07%) for the pelagic sharks and *Dollfusiella* (7.81%) and *Grillotia* (7.93%) for the benthic sharks contributed over 50% to the total dissimilarity. Other characteristic trypanorhynch genera of the benthic sharks were *Gilquinia*, *Prochristianella* Dollfus, 1946, *Diesingium* Pintner, 1929, *Deanicola* Beveridge, 1990 and *Pseudolacistorhynchus* Palm, 1995. The parasite genera whose species numbers significantly differed between pelagic and benthic sharks were *Tentacularia* (U = 171.0, p = 0.003), *Nybelinia* (U = 177.5, p = 0.025), *Heteronybelinia* (U = 193.0, p = 0.029), *Pseudogrillotia* Dollfus, 1969 (U = 216.0, p = 0.033), *Dasyrhynchus* (U = 144.0, p = 0.000), *Lacistorhynchus* (U = 195.0, p = 0.013), *Pseudolacistorhynchus* (U = 232.0, p = 0.034), *Callitetrarhynchus* (U = 180.0, p = 0.013), *Floriceps* (U = 180.0, p = 0.005), *Poecilancistrum* Dollfus, 1929 (U = 207.0, p = 0.020), *Otobothrium* (U = 155.0, p = 0.002), *Tetrarhynchobothrium* Diesing, 1854 (U = 217.5, p = 0.012) and *Dollfusiella* (U = 156.5, p = 0.001).

The average similarity and genus composition of trypanorhynchs for fish vs. invertebrate feeding sharks is compiled in Table S3. Fish-feeding sharks had a 19.38% average similarity, with the trypanorhynch genera *Nybelinia* (30.43%), *Grillotia* (14.98%), *Heteronybelinia* (10.53%), *Otobothrium* (9.89%), *Dasyrhynchus* (9.72%), *Callitetrarhynchus* (6.96%), *Tentacularia* (4.57%) and *Floriceps* (3.29%) having a contribution of 90.37%. Invertebrate-feeding sharks had an average similarity of 18.76%, and were characterized by the genera *Dollfusiella* (40.53%), *Nybelinia* (30.13%), *Lacistorhynchus* (8.27%), *Prochristianella* (4.29%), *Diesingium*, *Trigonolobium* Dollfus, 1929 and *Tetrarhynchobothrium* (together 9.66%). The decreasing contribution to the total similarity, up to 100%, is given in Table S3. The parasite genera whose species numbers significantly differed between fish and invertebrate-feeding sharks were *Tentacularia* (U = 126.5, p = 0.025), *Heteronybelinia* (U = 124.0, p = 0.036), *Kotorella* Euzet and Radujkovic, 1989 (U = 160.0, p = 0.044), *Dasyrhynchus* (U = 110.0, p = 0.009), *Grillotia* (U = 129.0, p = 0.048), *Paragrillotia* Dollfus, 1969 (U = 160.0, p = 0.044), *Lacistorhynchus* (U = 123.0, p = 0.007), *Pseudolacistorhynchus* (U = 144.0, p = 0.007), *Diesingium* (U = 128.0, p = 0.001), *Floriceps* (U = 132.0, p = 0.035), *Otobothrium* (U = 123.0, p = 0.034), *Tetrarhynchobothrium* (U = 128.0, p = 0.001), *Zygorhynchus* Beveridge & Campbell, 1988 (U = 160.0, p = 0.044), *Eutetrarhynchus* Pintner, 1913 (U = 149.5, p = 0.047), *Dollfusiella* (U = 74.5, p = 0.000), *Trigonolobium* (U = 144.0, p = 0.007), *Trimacracanthus* Beveridge and Campbell, 1987 (U = 160, p = 0.044) and *Prochristianella* (U = 133.0, p = 0.008).
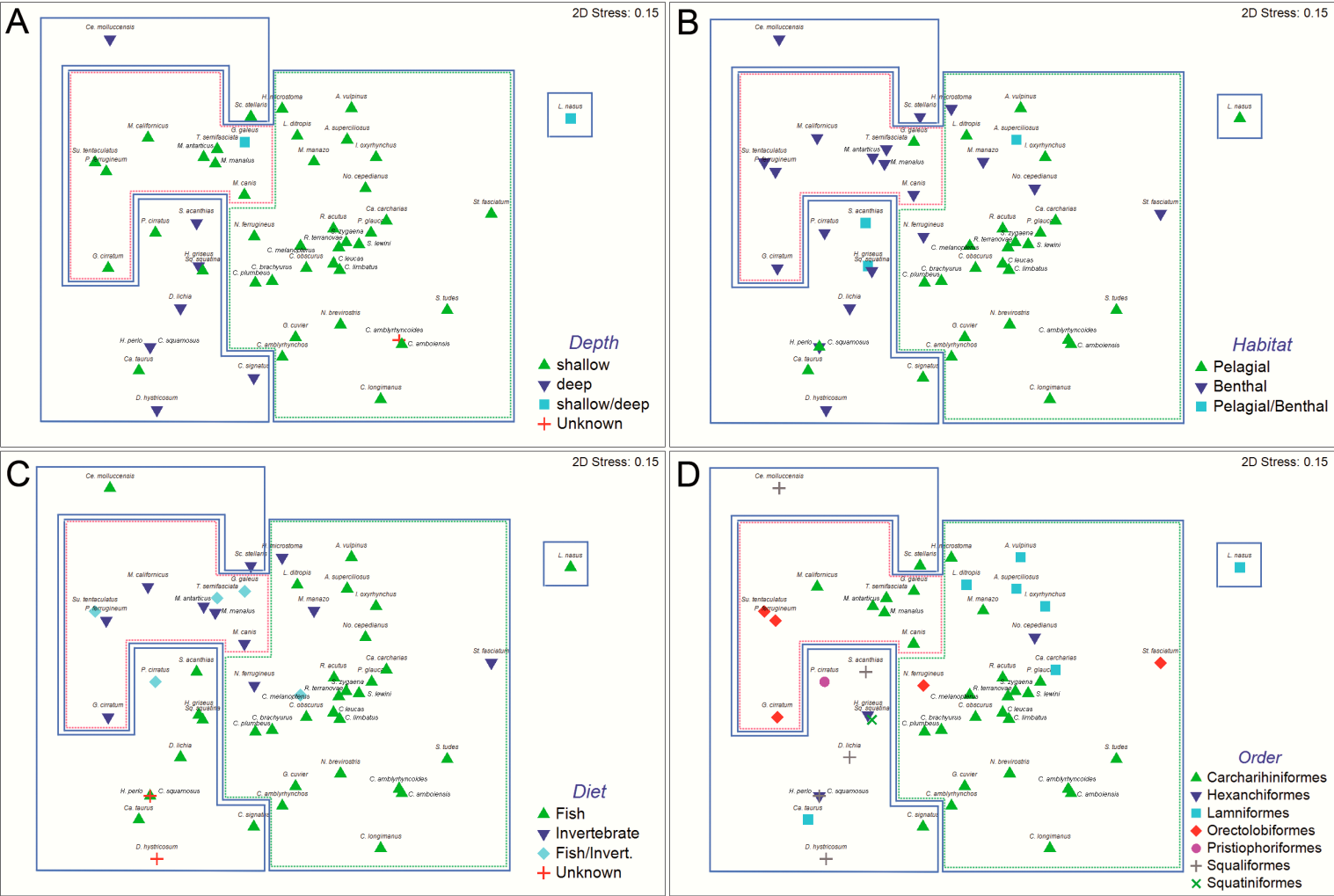
Figure 2. Multidimensional scaling plot for all shark species based on Bray-Curtis similarity and grouped by depth (**A**), habitat (**B**), diet (**C**), and order (**D**). Clusters I–III marked in blue, cluster IV in red, and cluster V in green.

The average similarity and genus composition of trypanorhynchs for fish vs. invertebrate feeding sharks is compiled in Table S3. Fish-feeding sharks had a 19.38% average similarity, with the trypanorhynch genera *Nybelinia* (30.43%), *Grillotia* (14.98%), *Heteronybelinia* (10.53%), *Otobothrium* (9.89%), *Dasyrhynchus* (9.72%), *Callitetrarhynchus* (6.96%), *Tentacularia* (4.57%) and *Floriceps* (3.29%) having a contribution of 90.37%. Invertebrate-feeding sharks had an average similarity of 18.76%, and were characterized by the genera *Dollfusiella* (40.53%), *Nybelinia* (30.13%), *Lacistorhynchus* (8.27%), *Prochristianella* (4.29%), *Diesingium*, *Trigonolobium* Dollfus, 1929 and *Tetrarhynchobothrium* (together 9.66%). The decreasing contribution to the total similarity, up to 100%, is given in Table S3. The parasite genera whose species numbers significantly differed between fish and invertebrate-feeding sharks were *Tentacularia* (U = 126.5, p = 0.025), *Heteronybelinia* (U = 124.0, p = 0.036), *Kotorella* Euzet and Radujkovic, 1989 (U = 160.0, p = 0.044), *Dasyrhynchus* (U = 110.0, p = 0.009), *Grillotia* (U = 129.0, p = 0.048), *Paragrillotia* Dollfus, 1969 (U = 160.0, p = 0.044), *Lacistorhynchus* (U = 123.0, p = 0.007), *Pseudolacistorhynchus* (U = 144.0, p = 0.007), *Diesingium* (U = 128.0, p = 0.001), *Floriceps* (U = 132.0, p = 0.035), *Otobothrium* (U = 123.0, p = 0.034), *Tetrarhynchobothrium* (U = 128.0, p = 0.001), *Zygorhynchus* Beveridge & Campbell, 1988 (U = 160.0, p = 0.044), *Eutetrarhynchus* Pintner, 1913 (U = 149.5, p = 0.047), *Dollfusiella* (U = 74.5, p = 0.000), *Trigonolobium* (U = 144.0, p = 0.007), *Trimacracanthus* Beveridge and Campbell, 1987 (U = 160, p = 0.044) and *Prochristianella* (U = 133.0, p = 0.008).

Only three trypanorhynch genera, mainly *Grillotia* with a 94.25% total similarity contribution, *Gilquinia* (4.24%), and *Nybelinia* (1.51%) characterised the deep water sharks (Table S4). The other trypanorhynch genera were characteristic of shallow water shark species occurring above 200 m depth. *Nybelinia* (39.30%), *Otobothrium* (10.10%) and *Dollfusiella* (9.60%) contributed most, followed by *Dasyrhynchus* (6.23%), *Callitetrarhynchus* (6.15%), *Heteronybelinia* (5.07%), *Grillotia* (3.99%), and *Tentacularia*, *Mixonybelinia* Palm, 1999, and *Lacistorhynchus* (together 10.20%) (Table S4). The parasite genera whose species numbers significantly differed between shallow and deep water sharks were *Nybelinia* (U = 85.0, p = 0.016), *Chimaerarhynchus* Beveridge and Campbell, 1989 (U = 136.5, p = 0.014), *Dasyrhynchus* (U = 108.0, p = 0.038), *Deanicola* (U = 136.5, p = 0.014), *Grillotia* (U = 34.5, p = 0.000), *Callitetrarhynchus* (U = 104.0, p = 0.030), *Dollfusiella* (U = 108.0, p = 0.037), and *Otobothrium* (U = 100.0, p = 0.025).

Similarity percentage analysis further revealed the importance of the different trypanorhynch genera for the characterisation of the shark orders (Table S5). The Carcharhiniformes were characterized by *Nybelinia* (33.03%), *Otobothrium* (13.43%), *Dasyrhynchus* (10.14%), *Callitetrarhynchus* (9.98%), *Heteronybelinia* (8.83%), *Dollfusiella* (4.90%), *Tentacularia* (4.67%), *Floriceps* (4.39%), and *Lacistorhynchus* (2.88%). The genera *Grillotia* and *Nybelinia* contributed 73.33% and 26.67% to the similarity of the Hexanchiformes, while the Lamniforms were characterised by *Nybelinia* (35.68%), *Sphyriocephalus* Pintner, 1913 (20.43%), *Mixonybelinia* (19.68%), *Gymnorhynchus* Rudolphi, 1819 (11.09%), and *Molicola* Dollfus 1935 (9.24%). The genera *Dollfusiella* (83.56%) and *Mixonybelinia* (9.86%) contributed most to the similarity of the Orectolobiformes, and *Grillotia* (86.11%) and *Gilquinia* (13.89%) to that of the Squaliformes (Table S5).

The genera *Grillotia* (15.18%), *Nybelinia* (11.69%), *Gilquinia* (9.24%), *Otobothrium* (7.92%), and *Dasyrhynchus* (6.19%) contributed over 50% to the total dissimilarity of Carcharhiniformes and Squaliformes, and *Dollfusiella* (12.66%), and *Pseudolacistorhynchus* (4.78%) were important to separate the Orectolobiformes from the Carcharhiniformes. The genera *Nybelinia* (13.36%), *Sphyriocephalus* (9.15%), *Otobothrium* (7.98%), *Mixonybelinia* (6.44%), *Dasyrhynchus* (6.32%), and *Gymnorhynchus* (5.45%) contributed over 50% to the dissimilarity of the Carcharhiniformes and the Lamniformes, while the Lamniformes differed from the Orectolobiformes by *Dollfusiella* (15.4%), *Nybelinia* (14.18%), *Sphyriocephalus* (10.78%), *Mixonybelinia* (8.21%), and *Pseudolacistorhynchus* (5.25%), and differed from the Squaliformes by *Grillotia* (18.81%), *Nybelinia* (14.46%), *Sphyriocephalus* (12.40%), and *Gilquinia* (11.00%). The genera *Grillotia* (25.87%), *Nybelinia* (12.26%), and *Otobothrium* (8.04%) contributed over 50% to the dissimilarity of the Carcharhiniformes and the Hexanchiformes. Other orectolobiform characteristic genera contributed less than 5% (*Hornelliella* Yamaguti, 1954, *Molicola*, *Hepatoxylon* Bosc, 1811). Only four genera, *Grillotia* (20.90%), *Dollfusiella* (16.76%), *Gilquinia* (11.88%)

and *Deanicola* (6.73%) contributed 58.02% to the dissimilarity between the Orectolobiformes and the Squaliformes. The parasite genera where the species numbers significantly differed in seven orders of shark were *Nybelinia* (H = 11.0, p = 0.044), *Mixonybelinia* (H = 13.8, p = 0.016), *Sphyriocephalus* (H = 15.9, p = 0.007), *Gilquinia* (H = 13.2, p = 0.020), *Molicola* (H = 13.3, p = 0.019), *Gymnorhynchus* (H = 13.5, p = 0.017), *Grillotia* (H =19.7, p = 0.002), *Pseudolacistorhynchus* (H = 18.4, p = 0.002), *Callitetrarhynchus* (H = 11.2, p = 0.004), *Otobothrium* (H = 11.1, p = 0.043), and *Dollfusiella* (H = 13.5, p = 0.018).

2.2. Carcharhiniformes

The result of the hierarchical, agglomerative classification (CLUSTER) of trypanorhynch assemblages of Carcharhiniformes, the order with the most comprehensive trypanorhynch dataset in Palm [6], is shown in Figure 3. Two major clusters of sharks at an arbitrary dissimilarity level of 82% were recovered (I and II). The nMDS plots of the classification, with sharks coded for family, diet, and habitat are shown in Figure 4. Analysis of similarities revealed significant differences in trypanorhynch assemblage composition between sharks that primarily consume invertebrates versus those that primarily consume teleosts (global R = 0.683; p < 0.01), and also between those from benthic and pelagic habitats (global R = 0.590; p < 0.01). Regarding taxonomy, however, ANOSIM pairwise comparisons, detected significant differences on trypanorhynch assemblages only between Carcharhinidae and Triakidae (global R = 0.678; p < 0.01) and between Sphyrnidae and Triakidae (global R = 0.748; p < 0.01). No significant differences were found between the trypanorhynch assemblages of any of the other eight pairs of carcharhiniform families.
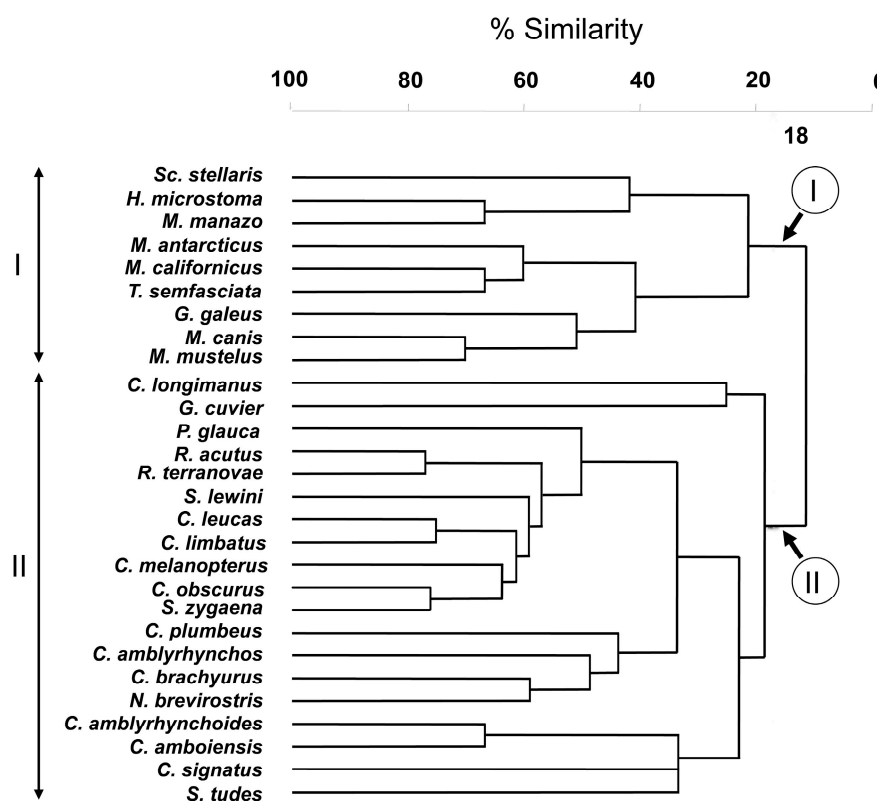

Figure 3. Cluster analysis of carcharhiniform sharks according to Trypanorhyncha assemblages. Roman letters I–II indicate the two major clusters.

The results of the SIMPER analyses to explore the contributions of individual trypanorhynch genera to the degree of similarity observed among the conditions for the factors environment and diet are shown in Tables S6 and S7, respectively. The average similarity was 31.16% for pelagic carcharhiniforms, with the genera *Nybelinia*, *Otobothrium* and *Dasyrhynchus* having a cumulative contribution of 52.53%. Together with *Heteronybelinia*, *Callitetrarhynchus*, *Tentacularia*, and *Floriceps*,

these trypanorhynch genera reached a cumulative contribution of 92.37%. Benthic carcharhiniforms that had an average similarity of 33.88% were characterized by four genera, *Dollfusiella*, *Lacistorhynchus*, *Nybelinia*, and *Diesingium*, having a cumulative contribution of 91.22%. Parasite genera whose species numbers significantly differed between pelagic and benthic carcharhiniforms were *Tentacularia* (U = 39.0, p = 0.031 one-tailed), *Heteronybelinia* (U = 33.0, p = 0.017), *Pintneriella* Yamaguti 1934 (U = 55.0, p = 0.028), *Dasyrhynchus* (U = 30.0, p = 0.012), *Lacistorhynchus* (U = 16.5, p = 0.000), *Diesingium* (U = 35.5, p = 0.002), *Floriceps* (U = 39.0, p = 0.031), *Otobothrium* (U = 37.5, p = 0.040), *Tetrarhynchobothrium* (U = 44.0, p = 0.002), *Eutetrarhynchus* (U = 47.0, p = 0.024), *Dollfusiella* (U = 19.0, p = 0.000), and *Trimacracanthus* (U = 55.0, p = 0.028).

Carcharhiniforms with different diet preferences, either fish or invertebrates, had an average similarity of 32.44 for fish and 29.47 for invertebrates. Similarly to the SIMPER analysis of benthic versus pelagic carcharhiniforms, *Nybelinia*, *Otobothrium*, and *Dasyrhynchus* reached a cumulative contribution close to 50% (48.95%) for preliminary fish eating sharks (Table S7). Together with *Heteronybelinia*, *Callitetrarhynchus*, *Tentacularia*, and *Floriceps*, the cumulative contribution was 91.43%. Invertebrate-feeding carcharhiniforms were characterized by the genus *Nybelinia*, that had a contribution of 50.24%, and together with *Dollfusiella*, *Lacistorhynchus*, *Diesingium*, and *Trigonolobium* reached a cumulative contribution of 91.92%. The decreasing contribution to the total similarity, up to 100%, is given in Table S7. The parasite genera whose species numbers were significantly different between fish and invertebrate feeders were *Tentacularia* (U = 35.0, p = 0.018), *Heteronybelinia* (U = 24.5, p = 0.004), *Gilquinia* (U = 45, p = 0.010), *Dasyrhynchus* (U = 24.5, p = 0.005), *Lacistorhynchus* (U = 27.0, p = 0.000), *Diesingium* (U = 36.0, p = 0.002), *Callitetrarhynchus* (U = 32.5, p = 0.019), *Floriceps* (U = 35, p = 0.018), *Poecilancistrum* (U = 42.0, p = 0.043), *Otobothrium* (U = 30.5, p = 0.016), *Tetrarhynchobothrium* (U = 45.0, p = 0.010), *Eutetrarhynchus* (U = 45.0, p = 0.010), *Dollfusiella* (U = 33.5, p = 0.008), and *Trigonolobium* (U = 45.0, p = 0.010).

Figure 4. Multidimensional scaling plot for carcharhiniform species based on Bray-Curtis similarity and grouped by habitat (**A**), diet (**B**), and family (**C**). Clusters I and II marked in blue.

The trypanorhynch composition within the shark family Carcharhinidae is characterised by the genera *Dasyrhynchus* (20.05% contribution), *Nybelinia* (14.52%), *Callitetrarhynchus* (13.90%), *Otobothrium* (12.61%), *Floriceps* (9.87%), *Heteronybelinia* (9.70%), *Tentacularia* (8.19%), and *Poecilancistrum* (3.96%) (Table S8). The Triakidae differed significantly from the Carcharhinidae and Sphyrnidae, being characterized by the genera *Lacistorhynchus* (31.15% contribution), *Nybelinia* (31.15%), *Dollfusiella* (23.20%), and *Diesingium* (9.10%). The Sphyrnidae harbored the genera *Otobothrium* (38.72%), *Heteronybelinia* (31.20%), and *Nybelinia* (22.56%). Only two genera, *Nybelinia* and *Trigonolobium*, were found in the Hemigaleidae, and *Nybelinia* and *Gilquinia* in the Scyliorhinidae. The parasite genera whose species numbers significantly differed in five families of carcharhiniforms were *Heteronybelinia* (H = 10.4, p = 0.017), *Gilquinia* (H = 12.6, p = 0.006), *Dasyrhynchus* (H = 10.5, p = 0.016), *Pseudogrillotia* (H = 8.3, p = 0.040), *Lacistorhynchus* (H = 21.9, p = 0.000), *Diesingium* (H = 13.4, p = 0.004), *Floriceps* (H = 9.6, p = 0.024), *Otobothrium* (H = 8.5, p = 0.037), *Dollfusiella* (H = 8.8, p = 0.033), and *Trigonolobium* (H = 14.5, p = 0.003).

3. Discussion

3.1. All Sharks

Our results demonstrate that depth distribution, diet composition and habitat type (benthic or pelagic) were the major influencing factors responsible for the composition of trypanorhynch assemblages and the groupings (cluster) of the sharks. Depth distribution affected the formation of all shark clusters (Figure 2): Cluster I included a single shallow/deep water species, Cluster II was dominated by deep water species, and Cluster III by shallow water species. Clusters IV and V were generated within Cluster III, and were dominated by invertebrate or teleost feeders, respectively. Cluster IV and Cluster V were also influenced by habitat type (benthic or pelagic), respectively. Moreover, Cluster II was dominated by piscivorous sharks. In general, no obvious pattern of the shark orders was found in the analysis. For instance, the most represented order Carcharhiniformes occurred in Clusters II and III (IV and V), and Lamniformes were found in Clusters I, II, and III (V).

Host-parasite associations are essential to gain information on the ecology of the hosts. The four shallow water shark species, *Squatina squatina*, *Pristiophorus cirratus*, *Carcharias taurus*, and *Scyliorhinus stellaris*, became an exception in Cluster II, because they were included in the deep water species group. That was probably due to the occurrence of *Nybelinia*, *Grillotia*, and *Gilquinia* that generally characterized the deep water species group, but which were also found in these four exception species. Thus, we can conclude that these four species which had been coded as shallow water types [12,13] might be collated to shallow/deep water shark species. Additionally, two shark species (*Deania hystricosa* and *Centrophorus squamosus*) that were part of Cluster II had been coded as unknown diet species. *Grillotia* that was found in both species characterized them as belonging into the fish feeder shark group. In Cluster III, *Carcharhinus amblyrhynchoides* was recognized as an unknown depth species. The trypanorhynchs *Heteronybelinia*, *Callitetrarhynchus* and *Dasyrhynchus* were found in this shark. Based on SIMPER analysis and Mann-Whitney U test, *Callitetrarhynchus* and *Dasyrhynchus* are characteristic trypanorhynchs of shallow water sharks. Hence, we consider *C. amblyrhynchoides* as a shallow water species. Three exception species were found in Cluster IV that was dominated by benthic and invertebratefeeding sharks, *Galeorhinus galeus*, *Triakis semifasciata* and *Sutorectus tentaculatus*. This exception leads us to suspect that the habitat type of *G. galeus* needs additional scrutiny; although Compagno [13] indicates this to be a pelagic species. Its trypanorhynch fauna suggests that it may be more benthic in its habits than currently recognized. From further analysis of the trypanorhynch assemblages we suggest that *G. galeus*, *T. semifasciata*, and *S. tentaculatus* prefer invertebrates over fish, although that had been coded as fish/invertebrate feeders.

In the last cluster V that was dominated by pelagic and piscivorous sharks, we found seven exception species. These were *Hymenolepis microstoma*, *Mustelus manazo*, *Notorynchus cepedianus*, *Stegostoma fasciatum*, *Nebrius ferrugineus*, *Alopias superciliosus*, and *Carcharhinus melanopterus*. *C. melanopterus* that had been coded as fish/invertebrate feeder seems to prefer more fish over invertebrates based on its trypanorhynch fauna. In *A. superciliosus* that was pelagic/benthic realm,

we found *Nybelinia*, *Mixonybelinia* and *Sphyriocephalus*. *Nybelinia*, and *Mixonybelinia* characterized both groups, pelagic and benthic environment, while *Sphyriocephalus* characterized the pelagic environment only. Hence, we conclude that *A. superciliosus*, where adult species of *Sphyriocephalus* can be found, prefers the pelagic habitat. We also noted that adult *Hepatoxylon* was found in *No. cepedianus* [6] which suggests this shark to live in a pelagic/benthic habitat, although coded as benthic [12]. *Hepatoxylon* is a sphyriocephalid trypanorhynch occurring in oceanic but also deep water hosts. Although *Sphyriocephalus* characterises pelagic species, the species number of *Sphyriocephalus* was not significant different between pelagic and benthic sharks. We found parasite genera that characterised the pelagic and benthic group in *M. manazo*, *S. fasciatum*, and *N. ferrugineus*. The parasite genera found in these three sharks also characterized fish and invertebrate feeder. We suspect the three shark species were changing from invertebrate feeder and benthic habitat to be fish/invertebrate feeder and occurring in the pelagic/benthic habitat.

The last exception species in cluster V was *H. microstoma*. Based on its trypanorhynch fauna, *H. microstoma* was a benthic and invertebrate feeder. It became part of the group that was dominated by pelagic and fish feeder species. A plausible reason for this was the genus *Nybelinia* that was found in *H. microstoma*. *Nybelinia* was found in pelagic and benthic habitat species, as well as fish and invertebrate feeder species. The species numbers of *Nybelinia* in pelagic species were significantly higher than the numbers in benthic species. However, this was not significantly different between fish and invertebrate feeders. Another genus found in *H. microstoma* was *Trigonolobium* (one species). The species numbers of *Trigonolobium* in invertebrate feeders was significantly higher than the numbers in fish feeders, but it was not significantly different between pelagic and benthic sharks. We conclude that based on *H. microstoma*, habitat was a more dominant factor than diet in generating the Clusters IV and V within Cluster III.

Several shark species, based on the trypanorhynch fauna, were differently characterized than known from the literature [12–15]. An obvious reason for this is the poor knowledge on many shark species. Another explanation can be that the shark species can changed their behavior due to environmental changes (e.g., availability of fish, temperature increase, less production). Sharks might change their diet with regard to their size and prey diversity [16,17]. It has been stated that sharks are one of the most affected species concerning habitat loss or habitat change caused by climate change [18], which could explain their habitat changes. Another factor causing difficult affiliation of the sharks is that the knowledge on the trypanorhynch fauna is also far from complete, with new host records and new species descriptions added year by year. In general, trypanorhynchs have low (second) intermediate host and high final host specificity [11]. Although many trypanorhynchs have higher final host specificity, when they change the intermediate host (e.g., from invertebrate to fish or from fish to other fish that has a different habitat) they have a high possibility to reach further final host. This leads to regular host switches that have been recognized in trypanorhynch phylogeny. When this situation happens, then the trypanorhynch fauna makes it more difficult to characterize the shark species and results in conflicting evidence.

3.2. Carcharhiniformes

Among the factors investigated in the carcharhiniform analysis, the relationship between habitat and trypanorhynch assemblage was most remarkable. In fact, with the exception of *G. galeus*, the two major clusters of sharks recovered from the CLUSTER analysis of trypanorhynch assemblage data exactly corresponded to habitat occupied by the sharks. However, *G. galeus* coded as a pelagic shark was identified as a benthic shark (see Discussion above).

nMDS suggests that the trypanorhynch fauna has substantial potential to indicate the habit type of their elasmobranch final hosts. However, it is interesting to explore this in more depth by considering the individual genera comprising those assemblages. The trypanorhynch fauna of pelagic carcharhiniform sharks is primarily characterised by species of the genera *Otobothrium*, *Dasyrhynchus*, *Heteronybelinia*, *Tentacularia*, and *Floriceps*. In contrast, the trypanorhynch fauna of benthic carcharhiniform sharks is primarily characterized by species of *Dollfusiella*, *Lacistorhynchus*, *Diesingium*, and *Eutetrarhynchus*. All nine of these genera can be considered to be useful

discriminatory taxa of environmental tendencies as the former five genera are abundant in pelagic sharks and scarce in benthic sharks while the latter four genera are scarce in pelagic sharks and abundant in benthic species. It should be noted that *Nybelinia* is abundant in sharks from both pelagic and benthic environments, as it occurs likewise in sharks and rays and, thus, it must be carefully used in combination with other taxa as a discriminatory taxon assigning the elasmobranch to a habit type. Thus a combination of tentaculariids, lacistorhynchids and otobothriids characterizes the Charcharhiniformes. According to Palm [6] these trypanorhynch taxa genera represent at least two different life-cycles. The intermediate hosts of tentaculariids, such as *Tentacularia* and *Nybelinia*, are pelagic or oceanic teleosts as well as squids as paratenic hosts. These trypanorhynchs develop robust plerocercoids in the intermediate host, and are characterised by low host specificity [11]. Lacistorhynchoids and otobothroids develop plerocerci with blastocysts, and the intermediate hosts are often pelagic fish. For example, the otobothriid *Otobothrium penetrans* mainly infests belonids as second intermediate hosts that are most common in the upper layer (10 m) of the water column [19,20]. Eutetrarhynchoids and gymnorhynchoids are generally missing within the pelagic carcharhiniforms. The former have life cycles that involve invertebrates as intermediate hosts, and are most commonly found on rajiform elasmobranchs, often in the benthic environment. The latter develop merocercoids and live in pelagic hosts.

The relationship between diet and trypanorhynch assemblage was similarly notable. Of the 28 species of carcharhiniform sharks included in the analysis, the diets of all but four species were coincident with the two major clusters of sharks recovered from the CLUSTER analysis of trypanorhynch assemblage data (Figure 4). Remarkably, these exceptions were the three species (*C. melanopterus*, *T. semifasciata*, and *G. galeus*) coded as consuming both fish and invertebrates based on the diet data available in Compagno [13]. These results suggest that fish play a larger role in the diet of *C. melanopterus*, than do invertebrates. They further suggest that the diets of *T. semifasciatea* and *G. galeus* emphasize invertebrates over fish.

At the individual genus level, *Otobothrium*, *Dasyrhynchus*, *Heteronybelinia*, *Callitetrarhynchus*, *Tentacularia*, and *Floriceps* appear to be excellent indicators of a preference for fish as they are particularly abundant in piscivorous sharks and rare to non-existent in sharks that consume invertebrates. In contrast, *Dollfusiella*, *Lacistorhynchus*, *Diesingium*, and *Trigonolobium* appear to be particularly significant in invertebrate feeders, and rare in piscivorous shark species. According to food preferences, the SIMPER analysis showed a similar picture. This is in line with the typical life cycle of the trypanorhynchs (see above). According to the trypanorhynch fauna, the studied carcharhiniforms of group 2, Carcharhinidae, and Sphyrnidae, obviously, are mainly pelagic fish feeders.

Carcharhiniforms that were separately clustered from the pelagic fish feeders were the Triakidae, Scyliorhinidae and Hemigaleidae. The benthic carcharhiniforms of group 1 are characterized by the trypanorhynch genera *Dollfusiella*, *Lacistorhynchus*, *Nybelinia* and *Diesingium*, while the invertebrate feeders were characterized by *Dollfusiella*, *Lacistorhynchus*, *Diesingium*, and *Trigonolobium*. The life cycle of the eutetrarhynchoid *Dollfusiella* is known to involve benthic invertebrates such as shrimps and crabs as second intermediate host, while the second intermediate host of *Trigonolobium* is yet unknown [6]. However, our results give a first indication that *Trigonolobium* might also involve marine invertebrates within its life cycle. The importance of *Lacistorhynchus* and *Diesingium* to distinguish this group of carcharhiniforms gives conflicting evidence. Both genera are typical trypanorhynchs from triakid sharks, with the plerocercus stage of the former often infecting a range of, mainly demersal or bottom-dwelling fish species. Thus, *Lacistorhynchus* and also the lacistorhynchid genus *Diesingium* might indicate the benthic environment of triakid carcharhiniform sharks, however, they cannot be utilized to analyze the feeding ecology of their hosts.

The two major clusters of sharks recovered from CLUSTER analysis were consistent with the carcharhiniform family. In no instance were species of the same family found in both clusters. In addition, the ANOSIM test showed that the trypanorhynch assemblage of the Carcharhinidae significantly differed from that of the Triakidae. The lack of significance between the Carcharhinidae

and the Scyliorhinidae and the Hemigaleidae is likely to be the result of the lack of power of this test caused by the analyzed dataset, as the analysis included only a single species in each of the latter families. However, an additional level of resolution emerges if the phylogenetic relationships among carcharhiniform families will be considered. Based on the relatively robust, albeit preliminary molecular study Vélez-Zuazo and Agnarsson [21] concluded that hammerhead sharks (Sphyrnidae) were grouped with whaler sharks (Carcharhinidae) in phylogenetic relationship. The largest cluster (Cluster II) resulting from the agglomerative classification consists solely of members of this clade of sharks. In addition, ANOSIM failed to detect a significant difference between the assemblages of the Sphyrnidae and the Carcharhinidae. This test would be interesting to explore further with additional sphyrnid species because it included 16 species of carcharhinid sharks, but only three of the nine species of Sphyrnidae.

4. Materials and Methods

To study the host-parasite relationships between the Trypanorhyncha and their elasmobranch hosts, trypanorhynch data were analyzed from ca. 4000 host/parasite records compiled by Palm [6]. Only those shark species for which at least two adult trypanorhynch records had been available, were included in the analyses (See Table S1). Based on this minimum criterion, the trypanorhynch data consisted of 307 host/parasite records for a total of 50 shark species representing seven of the eight selachian orders (Carcharhiniformes, Lamniformes, Hexanchiformes, Orectilobiformes, Pristiophoriformes, Squaliformes, and Squatiniformes). These data, however, conspicuously emphasized carcharhiniform sharks, with 207 of the host/parasite records belonging to 28 species of this most speciose shark order.

A matrix was generated by scoring each shark species according to the total number of species in each trypanorhynch genus reported from any of the shark species. The absence of a trypanorhynch genus from a shark species was scored 0 in the matrix. A resemblance matrix among shark species was generated from this matrix of trypanorhynch assemblages choosing the Bray-Curtis measure [22,23]. All further multivariate data analyses were performed using the PRIMER software (release 6, Primer-E Ltd. 6.1.11, Meadow View, Plymouth, UK; Clarke and Warwick 2001) [24]. Sharks were classified by hierarchical agglomerative cluster analysis of this resemblance matrix using the group-average linking method CLUSTER [23]. The results of these analyses were then ordinated using nMDS two-dimensional ordination plots of shark species which were generated from trypanorhynch assemblage data for each of the factors under consideration. These plots were produced from both the full matrix of data for the selachian analyses, and also from the more restricted matrix consisting exclusively of carcharhiniform shark data. In each case, the conditions of the factor under consideration were superimposed on the ordination plot for that factor by coding each shark species with a symbol representing the condition it exhibited. For each factor, ANOSIM [25,26] was performed to test significant differences among conditions of the factor as reflected by trypanorhynch assemblage. In instances in which significant differences were seen, SIMPER analyses [27] were performed to explore which trypanorhynch genera contributed most to these differences. In these analyses trypanorhynch genera were ordered according to decreasing contribution (%) to the total similarity observed. Genera accounting for high levels of similarity were determined to be good candidates as individual indicators.

For the analyses, each shark species was categorized for habitat (benthic or pelagic) and diet (primarily invertebrates and primarily teleosts). For the analysis of the Selachii, sharks were also coded for depth of distribution (below 200 m as deepwater, 200 m or less as shallow water species). In the carcharhiniform analyses taxonomy was assessed at the level of family; in the Selachii analyses taxonomy was assessed at the level of order? Sharks were categorized for these factors based on information published in the work of Compagno [12–15], because this provides detailed data for all of the target species. Shark taxonomic position at order and family levels was also based on Compagno [14]. Sharks were categorised for diet, habitat, and depth distribution for the Hexanchiformes, Squaliformes, and Squatiniformes [11], for the Carcharhiniformes [12], and for the Lamniformes, Orectilobiformes, and Pristiophoriformes [14]. Categorization by habitat type was

fairly straightforward as most shark species were either benthic or pelagic. Coding for depth was similarly unambiguous with the recorded depths of most sharks being either above, or below 200 m. Categorisation of sharks according to diet, however, was somewhat more ambiguous. While many sharks had a clear preference for either invertebrate or teleost prey, other species consumed both types of prey with some regularity. In such instances, sharks were coded as exhibiting both conditions of this factor. For the purpose of this study, two mutually exclusive conditions for this factor were chosen, based on the final intermediate hosts utilized by trypanorhynchs (i.e., invertebrates vs. teleosts).

Kruskal-Wallis test and Mann-Whitney U test were performed to compare the number of parasite species from each genus in each shark group [28,29]. The Kruskal-Wallis test was employed for more than two categories of sharks (e.g., selachian order; Carcharhiniformes, Lamniformes, Hexanchiformes, Orectilobiformes, Pristiophoriformes, Squaliformes, and Squatiniformes), and Mann-Whitney U test was employed for two categories of sharks (e.g., habitat; benthic and pelagic).

5. Conclusions

This study demonstrates that nMDS of the elasmobranch parasitic Trypanorhyncha Diesing, 1863, is a useful tool for investigating parasites as indicators of the host biology in marine ecosystems. Transmission through the marine food web (via predation) and an unambiguous identification in the final (sharks and rays) and intermediate (teleosts, other marine invertebrates) hosts allow conclusions to be made on the feeding biology of the host. The relationship between trypanorhynch assemblage and investigated factors for all sharks was complex, but we found that depth distribution, diet, and habitat type were the major influencing factors. The relationships within carcharhiniform species were more robust, especially if the factor habitat is considered. Overlapping host ecology was evident even in phylogenetically-distant hosts, demonstrating host switches and low host specificity in some of the trypanorhynch taxa. Considering that the underlying dataset is based on a host-parasite list from 2004 [6] and more species descriptions and host records are added continuously nMDS proves to be a powerful tool to attribute aspects of shark biology (phylogeny, habitat, depth distribution, diet) to rare and difficult-to-observe elasmobranch species in future.

Supplementary Materials: The following are available online at www.mdpi.com/link, Table S1: List of shark species analysed in the recent study; Table S2: SIMPER analysis results for similarities in each habitat of all sharks; Table S3: SIMPER analysis results for similarities in each diet of all sharks; Table S4: SIMPER analysis results for similarities in each depth of all sharks; Table S5: SIMPER analysis results for similarities in each order of all sharks; Table S6: SIMPER analysis results for similarities in each habitat of carcharhiniform; Table S7: SIMPER analysis results for similarities in diet of carcharhiniform; Table S8: SIMPER analysis results for similarities in each family of carcharhiniform.

Author Contributions: H.W.P. collected the data and together with U.P. designed and performed the analyses. I.Y. contributed figures and supported the SIMPER analyses and statistical tests. The paper was jointly written among authors.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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