Characterization of ecto- and endoparasite communities of wild Mediterranean teleosts by a metabarcoding approach

Mathilde Scheifler1*, Magdalena Ruiz-Rodríguez1, Sophie Sanchez-Brosseau1, Elodie Magnanou1, Marcelino T. Suzuki2, Nyree West3, Sébastien Duperron4, Yves Desdevises1

1 Sorbonne Université, CNRS, Biologie Intégrative des Organismes Marins, BIOM, Observatoire Océanologique, Banyuls/Mer, France, 2 Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotecnologies Microbiennes, LBKM Observatoire Océanologique, Banyuls/Mer, France, 3 Sorbonne Université, CNRS, Observatoire Océanologique de Banyuls, Banyuls/Mer, France, 4 CNRS, Muséum National d'Histoire Naturelle, Molécules de Communication et Adaptation des Micro-organismes, UMR7245 MCAM, Muséum National d'Histoire Naturelle, Paris, France

* scheifler@obs-banyuls.fr

Abstract

Next-generation sequencing methods are increasingly used to identify eukaryotic, unicellular and multicellular symbiont communities within hosts. In this study, we analyzed the non-specific reads obtained during a metabarcoding survey of the bacterial communities associated to three different tissues collected from 13 wild Mediterranean teleost fish species. In total, 30 eukaryotic genera were identified as putative parasites of teleosts, associated to skin mucus, gills mucus and intestine: 2 ascomycetes, 4 arthropods, 2 cnidarians, 7 nematodes, 10 platyhelminthes, 4 apicomplexans, 1 ciliate as well as one order in dinoflagellates (Syndiniales). These results highlighted that (1) the metabarcoding approach was able to uncover a large spectrum of symbiotic organisms associated to the fish species studied, (2) symbionts not yet identified in several teleost species were putatively present, (3) the parasitic diversity differed markedly across host species and (4) in most cases, the distribution of known parasitic genera within tissues is in accordance with the literature. The current work illustrates the large insights that can be gained by making maximum use of data from a metabarcoding approach.

Introduction

Parasites are extremely diverse and omnipresent in all environments, making this lifestyle one of the most successful on Earth [1]. Parasites are usually classified as ectoparasites or endoparasites, with or without direct contact with the external environment respectively [2]. Parasites also differ in their life cycle: some parasites require only one host to complete their life cycle (direct life cycle) while others need intermediate hosts (indirect life cycle), in which they change their morphology and biology (mobility, reproduction, . . .) [2]. Fish are well known to be parasitized by many eukaryotic organisms, unicellular or multicellular [3–6]. Fish gills and
skin, constantly in contact with the surrounding water, are exposed to ectoparasitic organisms. The mucus layer covering skin and gills might act as a first barrier against these putative pathogenic organisms [7]. Endoparasites colonize the internal organs or tissues of their hosts, such as the thoracic cavity, muscles or organs of the digestive and urinary tract [2]. Through their impacts on host fitness and on host interactions with the environment (competition, predation, behavioral changes), parasites play an essential role in population dynamics and ecosystems functioning [1,8–10].

Studying parasitological communities is tedious, as traditional surveys involve lots of steps and deep knowledge of often small and complex organisms. Traditionally, parasitological surveys are performed by host dissection to recover parasites, followed by identification under a microscope based on morphological criteria, mostly of the adult forms [11]. The morphological identification of parasites requires extensive taxonomic expertise and is very time consuming [12–13]. It is then difficult to perform exhaustive studies of parasitic communities considering all different hosts and all parasite life stages based solely on morphology (many parasites being of very small size or even microscopic). A lot of parasites, such as helminths or copepods, go through different life stages (i.e. cyst, egg and larval stages) [14–17] that are often difficult or nearly impossible to locate [18] and even if some of them can be detected, morphological differences are insufficient to differentiate between species from these development stages [19–20]. In addition, these surveys usually focus on a single class of parasites, ecto- or endoparasites [21–24] and sometimes even on specific genera [25–27]. However, new sequencing methods have emerged as having the potential to more effectively characterize parasitic communities and open new avenues for parasitological research.

The use of high-throughput sequencing methods, such as metabarcoding is now commonplace in many fields of biological research [28–30]. This technique has already revolutionized our understanding of microbial diversity [31–33] and its use is booming in the field of eukaryotic diversity [12–13,34–35]. Metabarcoding allows a more in-depth investigation of entire parasite communities [36–39], including all life stages. Tanaka et al. (2014) compared the helminthic diversity of wild rat intestines using a traditional and a metabarcoding approach. They highlighted that the metabarcoding approach is reliable and useful to investigate parasitic diversity and should allow more accurate identification of parasites than a traditional method. In assessing the number of reads per parasitic species, they also hypothesized about the life stage of several parasitic species present (eggs, sexually immature adults, . . . ) [37]. Moreover, this technique is faster than traditional methods, relatively easier to perform and sensitive enough to be performed in a high-throughput manner [11,40–41].

In this study, we characterized the parasitic communities associated to skin mucus, gill mucus and intestine of several wild Mediterranean teleost fish species, by analyzing the non-specific reads obtained during a metabarcoding survey of the bacterial communities. In a global study of fish associated microbiota [42], we employed 16S rDNA primers biased towards bacteria and archaea [43]. However, these primers that can also amplify the 18S rDNA from eukaryotic organisms [43–44], also yielded several thousand reads that corresponded to eukaryotes. We further investigated the identity of these eukaryotic sequences associated with skin mucus, gill mucus and intestine of several teleost fish species from the Bay of Banyuls-sur-Mer (Gulf of Lion, northwest Mediterranean, France). We focused on 13 species, representing 5 families, easily observable in the Mediterranean Sea and each presenting different lifestyles, behavior and diet. Our aims were to i) test whether our metabarcoding approach, initially targeting bacterial DNA, was able to detect, in addition to bacterial communities, a symbiotic eukaryotic community present on the host and ii) assess whether they could correspond to previously undetected parasites, compared to what is known in the literature. Eukaryotic sequences obtained in initially bacteria-targeting metabarcoding approaches could
be a cheap way to gain at least some knowledge about the hidden diversity of eukaryotic parasites.

**Materials and methods**

**Ethics statement**

The Observatoire Océanologique de Banyuls-sur-Mer holds the authorization for fishing and housing wild Mediterranean teleosts (Decision n°100/2019, Direction Interrégionale de la Mer Méditerranée). Wild fish were caught (see below for details) by a competent person and in accordance with the European Union Regulations concerning the protection and welfare of experimental animals (European directive 91/492/CCE).

**Fish sampling**

Thirteen teleost fish species were collected in June 2017 (42°29'4.618"N, 3°8',35.39"E) and in October 2017 (42°29'15.073"N, 3°7',49.688"E) in the Bay of Banyuls-sur-Mer (northwest Mediterranean, France) (Table 1). Between two and five teleost specimens were collected for each species (Table 1). A gill fishing net was placed overnight in Banyuls bay between 0 and 6 m deep. About 6 hours later, fish were collected dead on the net, handled with gloves and put into individual plastic bags right after collection from the net. They were immediately brought from the vessel to the laboratory for dissection. Three different tissues were collected per fish individual and put into sterile tubes: skin mucus, gill mucus (with two gill arches) were collected with a sterile spatula and scissors, and for species that did not have mucus on their skin, we collected a portion of skin close to the lateral line of the fish (around 3 cm$^2$) by using ethanol-rinsed scissors and tweezers; a fragment (between 3 and 5 cm long) of the posterior region

| Samples | Fish family | Skin mucus | Skin | Gill mucus | Intestine |
|---------|-------------|------------|------|------------|-----------|
| Diplodus annularis$^1$ | Sparidae | 5 | | 5 | 3 |
| Diplodus vulgaris$^4$ | Sparidae | 2 | | 5 | 2 |
| Gobius bucchichi$^{1,2,3}$ | Gobiidae | 3 | | 5 | 3 |
| Gobius cruentatus$^2$ | Gobiidae | 2 | | 2 | 2 |
| Gobius niger$^{3,4}$ | Gobiidae | 2 | | 3 | 3 |
| Oblada melanura$^{1,4}$ | Sparidae | 4 | | 5 | 3 |
| Pagellus bogaraveo$^1$ | Sparidae | 3 | | 4 | 2 |
| Pagellus erythrinus$^3$ | Sparidae | 5 | | 5 | 5 |
| Sarpa salpa$^4$ | Sparidae | 5 | | 5 | 3 |
| Serranus scriba$^2$ | Serranidae | 5 | | 5 | 3 |
| Scorpaena notata$^1$ | Scorpaenidae | 5 | | 5 | 4 |
| Spicara maena$^1$ | Sparidae | 4 | | 4 | 2 |
| Symphodus tinca$^1$ | Labridae | 5 | | 5 | 3 |
| **Total** | | 45 | 5 | 58 | 38 | 146 |

Sampling in 2017

$^1$June 21th

$^2$June 26th

$^3$July 18th and

$^4$October 4th.

https://doi.org/10.1371/journal.pone.0221475.t001
of the intestine was also sampled (depending on the size of fish specimens). Samples were frozen at -80˚C until DNA extraction.

As mentioned before, aquatic organisms are constantly in contact with the surrounding water. In skin mucus, gill mucus and intestine, not only symbionts and DNA from hosts were recovered but also what is present in the surrounding environment (food and free-living organisms) and most likely traces of DNA leaved by all organisms, reflecting their current or past presence (i.e. environmental DNA) [34]. In order to verify that our sampling techniques were appropriate for the identification of parasitic taxa specifically associated to fish species (i.e. not present in the surrounding water), seawater was collected using a sterile container at each sampling date next to the gill net fishing to act as negative controls. Two liters of seawater were filtered onto a 0.2 μm nitrocellulose filter (Pall Corporation, U.S.A). Filters of each sampling date were frozen at -80˚C until DNA extraction.

DNA extraction and amplification

DNA was extracted by using the Quick-DNA Fecal/Soil Microbe MiniPrep Kit (Zymo Research, Orange, California) following manufacturer’s instructions. Samples were frozen at -80˚C. PCR amplification was carried out in triplicate and performed using primers targeting the hypervariable V4-V5 region of the 16S rRNA gene: 515F-Y (5’-GTGYCAGCMGCCGCGGTAA) and 926R (5’-CGGYCAATTYMTTTRAGTTT) [43]. The PCR mix contained 1X KAPA 2G Fast Ready Mix (Sigma-Aldrich, France), 0.2 μl of each primer (concentration of 0.2 μM), 3.6 μl of ultrapure water and 1 μl of DNA in a final volume of 10 μl. After 3 min of initial denaturation at 95˚C, the following conditions were applied: 22 cycles of 95˚C for 45s (denaturation), 50˚C for 45s (annealing) and 68˚C for 90s (extension), with a final extension at 68˚C for 5 min. For each sample, three PCRs were performed in the same conditions, to increase the DNA quantity, but also to avoid bias due to each reaction. Then, the product of each PCR was run on a 1% agarose gel at 100V for 20 minutes in an electrophoresis chamber (BIO-RAD) to visualize the presence of high molecular weight DNA. The visualization was carried out in a GelMaxTM photodocumenter (UVP). When the DNA was visible in the gel, amplifications from the same sample were pooled. Individual barcode sequences were added to each mix during a second PCR. The second PCR mix contained 1X KAPA 2G Fast Ready Mix (Sigma-Aldrich, France), 0.5 μl of each barcode (Nextera Index Sequences in http://seq.liai.org/204-2/), 10.5 μl ultrapure water and 1 μl of DNA for a final volume of 25 μl. PCR conditions were as follows: initial denaturation at 98˚C for 30s, 8 cycles of 98˚C for 10s, 60˚C for 20s, 72˚C for 30s and a final extension at 72˚C for 2 min. Incubation (37˚C for 30min, 85˚C for 15 min) with USB ExoSAP-IT PCR Product Cleanup (Thermofisher, France) was then performed to degrade unincorporated primers. All PCR products were normalized with a 96 well SequlPrep Normalization Plate (Thermofisher, France) and the normalized amplicons were concentrated by using the Wizard SV Gel and PCR Clean up Kit (Promega, France) (final concentration around 8 ng/μl). Finally, the concentration of each DNA sample was measured using the Quant-iT™ PicoGreen (Thermofisher, France) and amplicons were sequenced using Illumina 2x250 MiSeq sequencing (FASTERIS SA, Switzerland). Samples were run in two sessions and divided randomly. All the categories (fish species and body parts) were represented in both runs.

Sequence analyses

Sequence analysis was realized using an in-house pipeline based on Needham et al., 2018 [44] and scripts in both USEARCH9 [45] and Qiime V.1.9.1 [46]. A shell script 18Sclean.sh (see S1 Text) was used to pre-treat demultiplexed forward and reverse reads. Basically, as Needham
and collaborators, we used the strategy of using non-overlapping reads to identify eukaryotic sequences among sequences amplified with the Parada and collaborator’s primers [43]. Unassembled reads, identified using PEAR v.0.9.6 [47], were quality trimmed using the fastq_filter option of usearch9. Sequences in fasta format with both reads for each sample were merged with an "N" between the forward and the reverse-complemented reads. Sequences names were modified to include a sample label. Samples from all reads were merged in a single fasta file. At this stage, primer sequences were removed and the reads were dereplicated. The number of singletons was determined and they were denoised using usearch9 -unoise with minsize = 1. Since denoising of concatenated sequences appear to yield a large proportion of singletons, denoised sequences were clustered in Operational Taxonomic Units (OTUs) using usearch9 -cluster_otus using a stringent radius of 0.75 and minsize = 1. OTU tables were generated by usearch9 -usearch_global using -id = 0.97.

All OTUs were identified using QIIME’s assign_taxonomy.py -m rdp and the "SILVA_132_QIIME_release/taxonomy/18S_only/99/majority_taxonomy_7_levels.txt" file distributed by the SILVA project [48–49]. Finally, this OTU table was filtered to remove fish ("D_12__Teleostei") OTUs. Some sequences were assigned to taxonomic groups but not to a specific genus due to the poor representation of eukaryotic symbionts within the SILVA database [50–51]. To alleviate this problem, we used the program Basic Local Alignment Search Tool (BLAST, [52]) to assign them to a described genus whenever possible. Sequences with more than 98% similarity to a genus were kept and assigned. If a sequence matched with two or more genera, we used the OTU classification to choose the appropriate genus (with at least 98% similarity). In the absence of support from the OTU classification, or in the case of less than 98% similarity, the sequences were not assigned more precisely and not used in the analysis. Finally, all sequences unassigned to any taxonomic group (only 0.4% of all reads) were excluded. The remaining OTUs were used to assess the total eukaryotic diversity.

Results

A total of 146 samples were collected from 59 fish specimens in the Bay of Banyuls-sur-Mer (Table 1). The species Scorpæna notata did not have any mucus on its skin, so we collected a portion of the skin. The skin was treated as skin mucus for further analyses. A total of 3,064,621 sequences of minimum 400 bp length were obtained for the 146 samples. Around 76.2% of these reads corresponded to Bacteria and Archaea, whereas the rest of the sequences were assigned as eukaryotes. We investigated 44,258 sequences in total (representing 6.1% of eukaryotic reads) as potentially fish-associated symbionts (mutualistic, parasitic and commensal eukaryotic organisms; 93.8% of eukaryotic reads are fish sequences). The mean number of reads of potential symbionts per sample is 303.2 (standard deviation = 591.9).

For each species and for each tissue, we computed the mean value of the proportions in the samples. All sequences assigned to fish were removed. Among Eukaryota, representatives of clades Opisthokonta and SAR (Stramenopiles, Alveolates and Rhizaria) were detected in all tissues (skin mucus, gills mucus and intestine) of all the fish species. They were the most abundant groups, representing 88.16 to 100% of eukaryote-affiliated reads (Fig 1). The opisthokonts are a broad group of eukaryotes, composed of fungi, Ichthyosporea and metazoans. All of these three groups are represented in our analyses, but metazoans are ubiquitous and the most abundant in most samples (Fig 1). Within the SAR group, alveolates were detected in all tissues. The superfamilies Stramenopiles, Rhizaria, Fungi and Ichthyosporea were mainly present on the external surfaces of fish (13.95% and 5.06% on average for skin mucus and gill mucus respectively compared to 1.53% for intestine) (Fig 1). The four other superfamilies, Haptophyta, Excavata, Archeoplastidia and Amoebozoa together accounted for < 10% of the sequences in
skin mucus and < 9% of the sequences in gill mucus. They were mostly absent from the intestinal samples, apart from supergroup Archaeplastida retrieved from *Salpa salpa*, which represented 6.1% of eukaryotic sequences from this fish species (Fig 1C).

As this study focuses on parasitic communities of teleost fish species, sequences belonging to non-parasitic organisms were removed from the analysis. Therefore, we have eliminated most of photosynthetic organisms (i.e. green algae, red algae and green plants (Archaeplastida), some diatoms and dinoflagellates) [53], common dietary items and free-living
organisms. Parasites likely coming from organisms eaten by fish were also discarded (such as from, very likely, plant or arthropod hosts). In order to determine parasitic genera associated to fish, bibliographic searches were carried out using PubMed, Google Scholar and google searches. For each parasitic genus, we used keywords: "parasite", "fish" / "fish species", "name of the parasitic genus". Finally, only parasitic genera that were present at least in two samples (among the 146 collected) and with a minimum of 10 reads were retained for further examination (S1 Table).

We identified 30 genera as potential parasites of teleosts, representing 24,274 reads (0.8% of total reads): 2 Ascomycota, 4 Arthropoda, 2 Cnidaria, 7 Nematoda, 10 Platyhelminthes, 4 Apicomplexa, and 1 Ciliophora (Table 2). None of these parasitic genera has been identified in water samples (S2 Table).

Despite the fact that no genus could be determined within the order Syndiniales in our study, this order was also retained because some species are known to parasitize fish [106]. The structure of the parasitic communities was further investigated for each fish species (Figs 2 and 3). For greater clarity, only parasitic taxa representing at least 0.5% of eukaryote reads (obtained for each species) were represented (Figs 2 and 3, Table 2). All fish species were associated to several parasitic genera but their distribution greatly differed among host species (Figs 2 and 3). The genera *Aspergillus* (Ascomycota), *Eimeria* (Apicomplexa) and *Goussia* (Apicomplexa), as well as the order Syndiniales (Dinoflagellata) were ubiquitous in all the analyzed teleost families (Figs 2 and 3, Table 2). However, some taxa seemed to be more group specific to some hosts. For example, sequences from *Chondracanthus* (Copepoda) were detected only on *Serranus scriba* (51.1% of putative parasitic sequences identified on *Serranus scriba*, Fig 3E). Similarly, sequences from *Lepeophtheirus* (Copepoda) and *Polylabris* (Platyhelminthes, Monogenea) were only found in *Symphodus tinca* (6.4%, Fig 3F) and *Diplodus annularis* (5.1%, Fig 2A) respectively. Moreover, 1625 reads (6.7% of potential sequences from parasites) belonging to the genera *Taeniacanthus* (Copepoda), *Lamellodiscus*, *Microcotyle*, *Polylabris* (Monogenea) and *Opisthorchis* (Digenea) were only detected within the fish family Sparidae (Fig 2). No parasitic genus was found specifically associated to the Gobiidae family.

Among the 31 parasitic taxa, 9 genera (*Caligus* (Copepoda), *Unicapsula* (Myxozoa), *Cystidicola* and *Aonchotheca* (Nematoda), *Accacoelium*, *Lecithochirium* (Platyhelminthes), *Theileria*, *Eimeria* and *Goussia* (Apicomplexa)) and the order Syndiniales were observed in all tissues (gills mucus, skin mucus and intestine). Only 4 genera were present in only one tissue: *Lepeophtheirus*, *Taeniacanthus* (Copepoda) and *Polylabris* (Monogenea) were only observed in skin mucus, whereas *Opisthorchis* (Digenea) was only associated to fish gills. The other 17 genera were detected in two tissues (Table 3). Overall, sequences from 4 supergroups were mainly found in skin mucus: Ascomycota (65.5% of sequences), Arthropoda (82.1%), Cnidaria (93.9%) and Dinoflagellata (91.6%) (S3 Table). Nematoda and Apicomplexa were mainly identified in fish intestine (85.7% and 77.8% respectively) whereas Platyhelminthes and Ciliophora in fish gills (S3 Table). Monogeneans and ciliates are the only taxa present in only two tissues (62.9% and 90.9% in gills mucus and 37.1% and 2.4% in skin mucus for monogeneans and ciliates, respectively) (S3 Table).

**Discussion**

In this study, we performed a detailed analysis of eukaryotic sequences that were recovered from a metabarcoding study that was initially targeting bacteria associated to different tissues of teleost fish. The approach indeed yielded around 44,258 eukaryotic sequences (around 1.4% of total reads) that potentially open a window to the diversity of parasites associated with fish species.
**Benefits of a metabarcoding approach**

It is not surprising that we obtained many eukaryotic sequences with this primer set, from a metabarcoding approach initially targeting bacterial DNA. Indeed, the primer set was optimized to be universal for Bacteria and Archaea, but it has been also used to study the eukaryotic diversity, since in silico tests by Parada *et al.* 2016 [43] reportedly showed 0 mismatches to 86% of all eukaryotic sequences in the SILVA Database SSU r123 database. We repeated the analysis with 75,000 eukaryotic sequences present in the SILVA_132_SSURef_NR9_9_13_12_17_opt.arb tree of the Silva project [48–49] using the probe_match and the match function of arb_edit. The results show that only about 2200 sequences (c.a. 3%) had mismatches to the

### Table 2. Presence (black square) or absence (white square) of eukaryotic parasitic taxa within fish teleost species.

| Phylum, Class | Genus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---------------|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|
| Ascomycota, Eurotiomycetes | *Aspergillus* [54–55] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Ascomycota, Dothideomycetes | *Cladosporium* [56] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Arthropoda, Copepoda | *Caligus* [57–58] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Arthropoda, Copepoda | *Chondracanthus* [59–60] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Arthropoda, Copepoda | *Lepeophtheirus* [61–62] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Arthropoda, Copepoda | *Taeniaeacanthus* [63–64] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Cnidaria, Myxozoa | *Kudoa* [65] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Cnidaria, Myxozoa | *Unicapsula* [66–67] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Acanthocheilus* [68] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Contracaecum* [69–70] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Cucullanus* [27,71] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Dichelyne* [26,72] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Gystidica* [73–74] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Chromadorea | *Hysterobilharzium* [75–76] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Nematoda, Enoplea | *Aonchotheca* [77–78] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Monogenea | *Lamellodiscus* [25,79] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Monogenea | *Microcotyle* [80–81] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Monogenea | *Polylabris* [82–83] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Cardiocephaloides* [84–85] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Skoulelia* [86–87] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Accacoelium* [88] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Rhiodiocotyle* [89–90] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Lecithochirium* [91–92] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Opisthorchis* [59,93] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Platyhelminthes, Digenea | *Diphtherostomum* [94–95] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Apicomplexa, Acanoidasida | *Theileria* [96–97] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Apicomplexa, Canoidasida | *Cryptosporidium* [98–99] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Apicomplexa, Canoidasida | *Eimeria* [100–101] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Apicomplexa, Canoidasida | *Goussia* [102–103] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Ciliophora, Oligohymenophorea | *Trichodina* [104–105] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |
| Dinoflagellata, Syndiniophyceae | Order Syndiniales | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] |

**Total number of parasitic taxa**

| 9 | 12 | 12 | 6 | 11 | 16 | 10 | 14 | 16 | 11 | 7 | 7 | 17 |

[1] [https://doi.org/10.1371/journal.pone.0221475.t002](https://doi.org/10.1371/journal.pone.0221475.t002)
forward, and 4000 (c.a. 5%) to the reverse primer, with some groups having mismatches with both. Those mismatches were not against very broad groups; many mismatches were of a weak character (G-T mismatches) and in cases where a mismatch was to a broader group, these groups were not known to be marine, or associated with fish and thus we do not have any evidence that those primers would be particularly biased against a specific fish parasite group and invalidate our results. Moreover, the same primers have been also recently used to study symbiotic relationships including those of eukaryotes [44]. This approach gave us the opportunity to characterize, in addition to bacterial communities, the diversity of parasites associated with different fish tissues. These results highlight the fact that there are probably data on eukaryotic parasites in bacterial metabarcoding projects that are as yet unexploited.

Our results reveal diverse eukaryotic communities within several teleosts fish species that are far more taxonomically complex than anticipated by the current literature. This indicates that a metabarcoding approach allows for a faster and more complete identification of all
parasites than traditional methods in parasitology. In the field of parasitology, description and identification of parasitic eukaryotic species are still dominated by morphological analyses [11]. Moreover, these studies generally focus on one or a few parasitic species of a specific phylum (e.g. arthropods, nematodes, copepods) [58,65,81,87,98]. In the past few years, gene metabarcoding approaches have been booming in the field of parasitology [37–38]. However, recent studies using this approach have focused mainly on characterizing a single compartment of

Fig 3. Eukaryotic parasitic community structure and distribution of each parasitic taxa within skin mucus, gills mucus and intestine for the fish families Gobiidae, Scorpaenidae, Serranidae and Labridae. Percentages of parasitic taxa are indicated for proportions greater than 5%. Prevalence (proportion of individuals infected by a parasite taxon) is provided as a percentage around the outer circle.

https://doi.org/10.1371/journal.pone.0221475.g003
biodiversity, including the nemabiome (or more broadly the helminthic community) of terrestrial species [39,107–109]. To our knowledge, this is the one of the first study to attempt to identify, without a priori, all parasitic eukaryotic taxa present on teleost fish species using a universal metabarcoding approach [110]. Even if this approach was initially intended to characterize the bacterial communities of some fish species, it has allowed us to detect parasitic species belonging to different phyla (Table 2) [29,35,41]. While inventories of parasitic eukaryotic species are generally limited to the study of macro-organisms or organisms visible with a stereomicroscope, the metabarcoding approach also provides access to a broader fraction of the parasitic diversity, including microorganisms, intracellular and intra-organellar parasites, and those that are difficult to identify using morphological criteria [29,39,111].

### Table 3. Distribution of the 31 parasitic taxa within host gills mucus, skin mucus and intestine.

| Phylum            | Class          | Genus       | Number of reads | Gills mucus | Skin mucus | Intestine |
|-------------------|----------------|-------------|-----------------|-------------|------------|-----------|
| Ascomycota        | Eurotiomycetes | Aspergillus | 13              | 15.4        | 84.6       | 0         |
| Ascomycota        | Dothideomycetes| Cladosporium| 16              | 50          | 50         | 0         |
| Arthropoda        | Copepoda       | Caligus     | 186             | 0.5         | 95.7       | 3.8       |
| Arthropoda        | Copepoda       | Chondracanthus| 43           | 90.7        | 9.3        | 0         |
| Arthropoda        | Copepoda       | Lepeophtheirus| 21        | 0           | 100        | 0         |
| Arthropoda        | Copepoda       | Taenia canthus| 12        | 0           | 100        | 0         |
| Cnidaria          | Myxozoa        | Kudoa       | 422             | 7.3         | 92.7       | 0         |
| Cnidaria          | Myxozoa        | Unicapsula  | 949             | 0.1         | 94.5       | 5.4       |
| Nematoda          | Chromadorea    | Acanthocheilus| 3542       | 0           | 26.3       | 73.7      |
| Nematoda          | Chromadorea    | Contraecuum | 2893            | 10.2        | 0          | 89.8      |
| Nematoda          | Chromadorea    | Cucullanus  | 922             | 2.1         | 0          | 97.9      |
| Nematoda          | Chromadorea    | Dichelyne   | 4581            | 0           | 7.4        | 92.6      |
| Nematoda          | Chromadorea    | Cystidicola | 334             | 7.2         | 29.9       | 62.9      |
| Nematoda          | Chromadorea    | Hysterothylicum| 13        | 0           | 23.1       | 76.9      |
| Nematoda          | Enoplea        | Aonchotheca | 168             | 1.2         | 38.7       | 60.1      |
| Platyhelminthes   | Monogenea      | Lamellodiscus| 982          | 72.1        | 27.9       | 0         |
| Platyhelminthes   | Monogenea      | Microcystyle| 359             | 83.6        | 16.4       | 0         |
| Platyhelminthes   | Monogenea      | Polylabris  | 262             | 0           | 100        | 0         |
| Platyhelminthes   | Digenea        | Cardiocephaloides| 856       | 93.7        | 0          | 6.3       |
| Platyhelminthes   | Digenea        | Skouleka    | 2401            | 99.7        | 0.3        | 0         |
| Platyhelminthes   | Digenea        | Accacoeleum | 432             | 99.1        | 0.7        | 0.2       |
| Platyhelminthes   | Digenea        | Rhipidocystyle| 293        | 98.3        | 1.7        | 0         |
| Platyhelminthes   | Digenea        | Lectithochirium| 408       | 74.8        | 23.8       | 1.4       |
| Platyhelminthes   | Digenea        | Opisthochiris| 10             | 100         | 0          | 0         |
| Platyhelminthes   | Digenea        | Diplotherostomum| 246       | 0.8         | 0          | 99.2      |
| Apicomplexa       | Aconoidasida   | Theileria   | 24              | 8.4         | 45.8       | 45.8      |
| Apicomplexa       | Conoidasida    | Cryptosporidium| 265        | 2.6         | 97.4       | 0         |
| Apicomplexa       | Conoidasida    | Eimeria     | 361             | 3.9         | 1.1        | 95        |
| Apicomplexa       | Conoidasida    | Goussia     | 834             | 3.7         | 0.4        | 95.9      |
| Ciliophor a       | Oligohymenophorea | Trichodina| 1972            | 98.7        | 1.3        | 0         |
| Dinoflagellata    | Syndiniophyceae|            | 454             | 7.9         | 91.6       | 0.5       |

Each line represents the proportion of reads of the parasitic organism in each tissue in relation to the number of total eukaryotic reads obtained for this taxa. The tissue with the largest percentage of each genus is in bold.

[https://doi.org/10.1371/journal.pone.0221475.t003](https://doi.org/10.1371/journal.pone.0221475.t003)
However, the metabarcoding approach applied to parasitic communities is still subject to limitations and some aspects need to be improved [39,108]. First, this approach allowed us to identify 24274 reads originating from potential parasites of teleosts among 146 samples. Some parasite genera have a large number of reads (Acanthocheilus, Contracaecum, . . .) while others have only a few dozen, such as Aspergillus or Cladosporium (Ascomycota). However, none of the parasitic genera identified in fish tissues were found in water samples. It reinforces the claim that there was no contamination among our samples and that the reads we identified do not correspond to eDNA [34] and are well associated with the different fish tissues. Even if this approach provides reliable information on the presence of parasitic species on a host organism [39,35,41], the presence of a species with a small number of reads should be taken cautiously. In addition, none of these data should be considered quantitative [112]. Parasitic load is defined as the number of parasites in a host individual and it can be measured directly by dissecting organisms to count the number of adult parasites of each species [113] or indirectly by quantifying the number of parasitic eggs in faeces, but this number is not necessarily related to the number of parasitic individuals in the host [39,114]. Moreover, in high-throughput sequencing technologies, many technical factors (DNA extraction, PCR primers suspected of amplifying the DNA of some species at the expense of others, and sequencing technique) but also biological factors (such as the amount of DNA that varies according to the species, size and stage of the individuals) can influence the number of times a sequence is observed [108,115]. Metabarcoding can assess a larger fraction of the biodiversity but abundances obtained from sequencing cannot be interpreted as with morphology-based methods, since it does not reflect quantitative data [13,112]. As is already the case in the microbial domain, methods must be developed to obtain and interpret quantitative data from high throughput sequencing of eukaryotic symbiotic communities [109,116]. In addition, high throughput sequencing may not be used to identify all species [35,41,107]. Indeed, reference databases for taxonomic assignment are mostly incomplete [50–51,117]. In this study, we used the SILVA 132 database, but despite regular updates [118], some sequences could not be assigned and, because they were in very low numbers, they were not included in our analysis. In the coming years, more taxa will be added to databases, allowing the scientific community to use the full potential of metabarcoding approaches to characterize broader symbiotic communities [107,119–120].

**Distribution of potentially parasitic genera**

In this study, 30 genera (distributed among 7 phyla) were identified as potential parasites of skin mucus, gills mucus and intestine of 13 wild Mediterranean teleost fish species. Their distribution greatly differed among host species: 6 to 17 parasitic taxa were identified depending on the fish species (Table 2). In total, based on the 30 parasitic genera found, we listed 137 fish-parasite associations (Table 2). To our knowledge and according to the literature, 89 of these associations were new to the field of parasitology (see S2 Text). In most cases, the distribution of the different parasitic genera in the different fish tissues (skin mucus, gills mucus and intestine) is consistent with what has been described in the literature, for fungi, copepods, cnidarians, monogeneans, ciliates and most of the Apicomplexa. Some differences were nevertheless found for some parasitic taxa, in particular for nematodes and Digenea. As expected, the distribution of all monogenean genera in skin mucus and gills mucus is consistent with their life cycle [8,14]. Monogeneans are hermaphroditic and predominantly oviparous (including all genera identified here). The adults release eggs, from which ciliated larvae (oncomiracidium) hatch and swim freely. These larvae are attracted by fish mucus and colonize their skin [121–123]. Oncomiracidia then loose their ciliature and migrate from the skin to the gills of
the host [122,124–125]. It is therefore not surprising to find monogeneans on both gills (adult organisms) and skin (larvae) of their sparid hosts.

Within the class Digenea, three genera, *Lecithochirium*, *Diphtherostomum* and *Cardiocephaloides*, differed slightly from previous studies. Indeed, *Cardiocephaloides* had only been identified previously in skin and gill mucus [84–85] and the two other genera were reported only in organs of the digestive system and it is not the case in our study. Several hypotheses can be formulated to explain this distribution. The digenean life cycle is complex and composed of different stages, typically with two intermediate hosts. When eggs hatch (released from adult worms present on the definitive host), they release free-swimming larvae (miracidia) that detect a first intermediate host and penetrate its tissues and skin [126–127]. Different life stages then develop in this host: sporocysts and/or rediae, which in turn release the cercarial stage, a free-swimming organism which infect the second intermediate host. It is therefore not surprising to find digenean parasites on the surface’s tissue of their hosts (skin and gills). Alternatively, the internal anatomy of teleost fishes could also have been a factor, particularly the position of the intestine which begins at the posterior edge of the gills and ends at the anus [128]. Due to its location, the intestine may have contaminated other organs, including the gills and skin.

While all parasitic genera belonging to the phylum Nematoda should be identified in or on the internal organs of their fish hosts, such as the intestine, some genera were identified on the skin mucus (*Acanthocheilus*, *Dichelyne*, *Cystidicola*, *Hysterothyacium* and *Aonchotheca*) and also on gills (*Contracaecum*, *Cucullanus*, *Cystidicola* and *Aonchotheca*). Among parasitic nematodes, some have an indirect life cycle, which means that they need several successive hosts to complete their life cycle and reach the adult stage [70,76,129]. This is the case for seven genera of nematodes found in the present study, identified on the gills or skin of fish (Table 3): the intermediate host is usually a crustacean (isopods, amphipods, copepods, decapods) and the final host is a teleost [69,76,130–134]. Sequences identified on the skin mucus or on gills may then correspond to larval stages or eggs of nematodes, contained in crustaceans that could have been on the fish’s surfaces.

**Conclusion**

In this study, we analyzed the eukaryotic sequences of a universal gene metabarcoding approach, initially targeting bacterial DNA, in order to identify potential endo- and ectoparasitic eukaryotic species associated with several wild Mediterranean teleost fish species. We illustrated the strong potential of this approach to reveal and identify an unexpectedly large spectrum of eukaryotic parasites within a host and therefore significantly increase our knowledge about the distribution of these organisms. The results obtained are consistent, for the most, with the literature, which highlights that data sets from metabarcoding approaches should therefore be used to the maximum, even beyond what they were originally intended for, as they can give us an overview of other communities in the studied environment. Thanks to this approach, we were able to detect a total of 30 parasitic genera distributed among 7 different phyla. This study also highlighted that each host has a different parasitic diversity. Despite its limitations, the metabarcoding approach provides a powerful, fast and readily available toolbox that could be applied for parasitological surveys. In general, DNA metabarcoding has an enormous potential to increase data acquisition in biodiversity surveys and therefore deliver key information to address many fundamental and applied research questions in ecology.

**Supporting information**

S1 Text. Shell script 18Sclean.sh.

(WORD)
S2 Text. Fish-parasite associations known from the literature.
(DOCX)

S1 Table. Presence (black square) or absence (white square) of the eukaryotic parasitic taxa within fish teleost species with less than 10 reads.
(DOCX)

S2 Table. OTU table corresponding to water samples (negative controls at each sampling date).
(XLSX)

S3 Table. Distribution of different parasitic phyla within gills mucus, intestine and skin mucus. Each line represents the proportion of reads of the parasitic phyla in each tissue in relation to the number of total eukaryotic reads obtained for this taxa. The largest percentage for each phylum is in bold.
(DOCX)

Acknowledgments
This work was funded by Sorbonne Université, programme Emergence 2016 (project SU-16-R-EMR-22-MICROFISH). We would like to thank the Bio2Mar platform (http://bio2mar.obs-banyuls.fr) for the access to the instrumentation and all the advice given to carry out this project, as well as the “Service des Moyens à la Mer (FR3724) de l’Observatoire Océanologique de Banyuls/Mer” for the fish sampling. Finally, we thank Isabelle Florent for sharing her knowledge of parasitology.

Author Contributions
Conceptualization: Sébastien Duperron, Yves Desdevises.
Formal analysis: Mathilde Scheifler, Marcelino T. Suzuki.
Funding acquisition: Sébastien Duperron, Yves Desdevises.
Investigation: Mathilde Scheifler, Magdalena Ruiz-Rodríguez, Nyree West.
Methodology: Marcelino T. Suzuki, Nyree West, Sébastien Duperron, Yves Desdevises.
Project administration: Yves Desdevises.
Supervision: Yves Desdevises.
Visualization: Mathilde Scheifler.
Writing – original draft: Mathilde Scheifler, Sébastien Duperron, Yves Desdevises.
Writing – review & editing: Mathilde Scheifler, Magdalena Ruiz-Rodríguez, Sophie Sanchez-Brosseau, Elodie Magnanou, Marcelino T. Suzuki, Nyree West, Sébastien Duperron, Yves Desdevises.

References
1. Lafferty K, Allesina S, Arim M, Briggs C, De Leo G, Dobson A, et al. Parasites in food webs: The ultimate missing links. Ecology Letters. 2008; 11(6):533–46. https://doi.org/10.1111/j.1461-0248.2008.01174.x PMID: 18462196
2. Bush AO, Fernández JC, Esch GW, Seed JR. Parasitism: The diversity and ecology of animal parasites. In: Parasitology; 2001.
3. Bahri S, Marques A. Gill infection of *Symphodus tinca* by *Henneguya* sp. (Myxozoa, Myxobolidae) in Kerkennah Islands, Tunisia. Bulletin of the European Association of Fish Pathologists. 2014; 28 (2):42–5.

4. Skovgaard A, Meneses I, Angelico MM. Identifying the lethal fish egg parasite *Ichthyodinium chabellardi* as a member of Marine Alveolate Group I. Environmental Microbiology. 2009; 11(8):2030–41. https://doi.org/10.1111/j.1462-2920.2009.01924.x PMID: 19453613

5. Boualleg C, Ferhati H, Kaoouchi N, Bensouilah M, Ternengo S. The copepod parasite of the gills of four teleost fishes caught from the gulf of Annaba (Algeria). African Journal of Microbiology Research. 2010; 4(9):801–7.

6. M'Rabet C, Ensibi C, Dhaouadi R, Yahia OK. A preliminary study on gill parasites of gilthead seabream *Sparus aurata* (Linnaeus 1758) (Pisces: Teleostei) from the eastern Tunisian sea-cage aquaculture. GERF Bulletin of Biosciences. 2016; 7(1):1–5.

7. Boutin S, Sauvage C, Bernatchez L, Audet C, Derome N. Inter individual variations of the fish skin microbiota: Host genetics basis of mutualism? PLoS ONE. 2014; 9(7):1–17.

8. Rohde K. Marine parasitology. CSIRO; 2005. 565 p.

9. Korallo NP, Vinarski M V., Krasnov BR, Mouillot D, Poulin R. Are there general rules governing parasite diversity? Small mammalian hosts and gamasid mite assemblages. Diversity and Distributions. 2007; 13(3):353–60.

10. Hatcher MJ, Dick JTA, Dunn AM. Diverse effects of parasites in ecosystems: Linking interdependent processes. Frontiers in Ecology and the Environment. 2012; 10(4):186–94.

11. Hino A, Maruyama H, Kikuchi T. A novel method to assess the biodiversity of parasites using 18S rDNA Illumina sequencing; parasitome analysis method. Parasitology International. 2016; 65(5):572–5.

12. Cowart DA, Pinheiro M, Mouchel O, Maguer M, Grall J, Mine E, et al. Metabarcoding Is Powerful yet Still Blind: A Comparative Analysis of Morphological and Molecular Surveys of Seagrass Communities. Mazzuca S, editor. PLOS ONE. 2015 Feb 10; 10(2):e0117562. https://doi.org/10.1371/journal.pone.0117562 PMID: 25668035

13. Lanzen A, Lekang K, Jonassen I, Thompson EM, Troedsson C. High-throughput metabarcoding of eukaryotic diversity for environmental monitoring of offshore oil-drilling activities. Molecular Ecology. 2016 Sep; 25(17):4392–406. https://doi.org/10.1111/mec.13761 PMID: 27454455

14. Lambert A. Oncomiracidiums et phylogénèse des Monogenea (Plathelminthes). Annales de Parasitologie Humaine et Comparée. 1980; 55(2):165–98. French PMID: 7458157

15. Martin JW, Olesen J, Høeg J. Atlas of crustacean larvae. 2014. 370 p.

16. Barson M. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in the catfish *Clarias gariepinus* (Burchell) from Lake Chivero, Zimbabwe. The Onderstepoort journal of veterinary research. 2004; 71(1):35–9 PMID: 15185573

17. Galindo GM, Rodrigues RA, Marcondes SF, Soares P, Tavares LER, Fernandes CE. Morphological and morphometric features of nematode-eyts in *Gymnotus inaequabilis* liver in the Brazilian Pantanal. Revista Brasileira de Parasitologia Veterinária. 2017; 26(3):285–91. https://doi.org/10.1590/S1899-2861201701044 PMID: 2892262

18. Trujillo-González A, Constantinoiu CC, Rowe R, Hutson KS. Tracking transparent monogenene parasites on fish from infection to maturity. International Journal for Parasitology: Parasites and Wildlife. 2015; 4(3):316–22. https://doi.org/10.1016/j.ijppaw.2015.06.002 PMID: 26199875

19. Laakmann S, Gerds T, Knebelsberger T, Martínez Arbizu P, Raupach MJ. Comparison of molecular species identification for North Sea calanoid copepods (Crustacea) using protomere fingerprints and DNA sequences. Molecular Ecology Resources. 2013; 13(5):862–76. https://doi.org/10.1111/1755-0998.12139 PMID: 23848968

20. Friedheim S. Comparison of Species Identification Methods DNA Barcoding versus Morphological Taxonomy. Mānoa Horizons. 2016; 1:74–86.

21. Rohde K, Hayward C, Heap M, Gasper D. A tropical assemblage of ectoparasites: gill and head parasites of *Lethrinus miniatus* (Teleostei, Lethrinidae). International Journal for Parasitology. 1994 24 (7):1031–53.

22. Rohde K, Hayward C, Heap M. Aspects of the ecology of metazoan ectoparasites of marine fishes. International Journal for Parasitology. 1995 Aug 1; 25(8):945–70. https://doi.org/10.1016/0166-8791(95)00015-4 PMID: 8550295

23. Ghiraldelli L, Lateca Martins M, Tomás Jeronimo G, Maia Yamashita M, de Barros Adamante W. Ectoparasites Communities from *Oreochromis niloticus* Cultivated in the State of Santa Catarina, Brazil. Journal of Fisheries and Aquatic Science. 2006; 1(2):181–90.
24. Ramdane Z, Trilles JP, Mahé K, Amara R. Metazoan ectoparasites of two teleost fish, Boops boops (L.) and Mullus barbatus barbatus L. from Algerian coast: Diversity, parasitological index and impact of parasitism. Cybium. 2013; 37(1–2):59–66.

25. Diamanka A, Boudaya L, Toguebaye BS, Parisse A. Lamellodiscus euzeti n. sp. (Monogenea: Diplectanidae), a parasite from Dentex canariensis and D. gibbosus (Teleostei: Sparidae) in the Atlantic Ocean and Mediterranean Sea. Parasite. 2011; 18(2):145–50. https://doi.org/10.1051/parasite/2011182145 PMID: 21279559

26. Moravec F, Justine J Lou. Cucullanid nematodes (Nematoda: Cucullanidae) from deep-sea marine fishes off New Caledonia, including Dichelyne etelidis n. sp. Systematic Parasitology. 2011; 78(2):95–108. https://doi.org/10.1007/s11230-010-9281-8 PMID: 21279559

27. Vieira FM, Pereira FB, Pantoja C, Soares IA, Pereira AN, Timi JT, et al. A survey of nematodes of the genus Cucullanus Müller, 1777 (Nematoda, Seuratoidei) parasitic in marine fishes off Brazil, including description of three new species. Zootaxa. 2015; 4039(2):289–311. https://doi.org/10.11646/zootaxa.4039.2.5 PMID: 26624480

28. Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, et al. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology. 2017; 26(21):5872–95. https://doi.org/10.1111/mec.14350 PMID: 28921802

29. Lobo J, Shokralla S, Costa MH, Hajibabaei M, Costa FO. DNA metabarcoding for high-throughput monitoring of estuarine macrobenthic communities. Scientific Reports. 2017 Dec 15; 7(1):15618. https://doi.org/10.1038/s41598-017-15823-6 PMID: 29142319

30. Smith KF, Kohli GS, Murray SA, Rhodes LL. Assessment of the metabarcoding approach for community analysis of benthic-epiphytic dinoflagellates using mock communities. New Zealand Journal of Marine and Freshwater Research. 2017; 51(4):555–76.

31. Zimmermann J, Glöckner G, Jahn R, Enke N, Gemeinhörzer B. Metabar-coding vs. morphological identification to assess diatom diversity in environmental studies. Molecular Ecology Resources. 2015 May 1; 15(3):526–42. https://doi.org/10.1111/1755-0998.12336 PMID: 25270047

32. Leung TLF, Donald KM, Keeney DB, Koehler AV., Peoples RC, Poulin R. Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: Life cycles, ecological roles and DNA barcodes. New Zealand Journal of Marine and Freshwater Research. 2009; 43(4):857–65.

33. Ruiz-Rodríguez M, Scheffler M, Sanchez-Brosseau S, Magnanou E, West N, Suzuki M Tet al. Host species and body site explain the variation in the microbiota associated to wild sympatric Mediterranean teleost fishes. Microbial Ecology. 2019 submitted
43. Parada AE, Needham DM, Fuhrman JA. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology. 2016; 18(5):1403–14. https://doi.org/10.1111/1462-2920.13023 PMID: 26271760

44. Needham DM, Fichot EB, Wang E, Berdjeb L, Cram JA, Fichot CG, et al. Dynamics and interactions of highly resolved marine plankton via automated high frequency sampling. The ISME Journal: Multidisciplinary Journal of Microbial Ecology. 2018 Jan 1; 12(10):2417–32.

45. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26 (19):2460–1. https://doi.org/10.1093/bioinformatics/btq461 PMID: 20709691

46. Caporaso JG, Justin Kuczynski, Stombaugh J, Bittinger K, Bushmann F, Costello E, et al. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 2010; 7(5):335–6. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131

47. Zhang J, Kober K, Flouri T, Stamatakis A. PEAR: A fast and accurate Illumina Paired-End read mergeR. Bioinformatics. 2010; 30(5):614–20. https://doi.org/10.1093/bioinformatics/btp593 PMID: 24142950

48. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research. 2013 Jan; 41(Database issue):D590–6. https://doi.org/10.1093/nar/gks1219 PMID: 23193283

49. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and “all-species Living Tree Project (LTP)” taxonomic frameworks. Nucleic Acids Research. 2014; 42(Database issue):643–8. https://doi.org/10.1093/nar/gkt888

50. Werner JJ, Koren O, Hugenholz P, DeSantis TZ, Walters WA, Caporaso JG, et al. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. The ISME journal: International Society for Microbial Ecology. 2012 Jan; 6(1):94–103.

51. Balvočiūtė M, Hudson DH. SILVA, RDP, Greengenes, NCBI and OTT—how do these taxonomies compare? BMC Genomics. 2017; 18(Suppl 2):114. https://doi.org/10.1186/s12864-017-3501-4 PMID: 28361695

52. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of Molecular Biology. 1990; 215(3):403–10. https://doi.org/10.1016/S0022-2836(05)80360-2 PMID: 2231712

53. Gómez F, Skovgaard A. A Parasite of Marine Rotifers: A New Lineage of Dinokaryotic Dinoflagellates (Dinophyceae). Journal of Marine Biology. 2015(1).

54. Chauhan R, Lone S. Pathogenicity of three species of Aspergillus (A. fumigatus, A. niger & A. sydowii) on some fresh water fishes. Life Sciences Leaflets. 2014; 48:65–72.

55. Tayyab M, Gul S, Nazir R, Rehman H, Rehman A, Saeed K, et al. Fish Parasites prevailing in the fishes of Indus River at D.I. Khan Khyber Pakhtunkhwa, Pakistan. Journal of Entomology and Zoology Studies. 2017; 5(4):422–7.

56. Tasic S, Miladinovic Tasic N. Cladosporium ssp.—Cause of Opportunistic Mycoses. Acta Fac Med Naiss. 2007; 24(1):15–9.

57. Ho JS, Lin CL. Three species of Caligus Müller, 1785 (Copepoda: Caligidae) parasitic on Caranx spp. (Teleostei: Carangidae) off Taiwan. Systematic Parasitology. 2007; 68(1):33–43. https://doi.org/10.1007/s11230-006-9084-0 PMID: 17429578

58. Ho J, Lin C, Chang W. Four species of Caligus Müller, 1785 (Copepoda, Siphonostomatoida, Caligidae) parasitic on marine fishes of Taiwan. Journal of Natural History. 2007; 41(5–8):401–17.

59. Andrews M, Battaglene S, Cobcroft J, Adams M, Noga E, Nowak B. Host response to the chondracanthid copepod Chondracanthus goldsmidi, a gill parasite of the striped trumpeter, Latris lineata (Forster), in Tasmania. Journal of Fish Diseases. 2010; 33(3):211–20. https://doi.org/10.10111/j.1365-2761.2009.01107.x PMID: 19912458

60. Braicovich PE, Lanfranchi AL, Incorvaia IS, Timi JT. Chondracanthid copepod parasites of dories (Zeiformes: Zeidae) with the description of a new species of Chondracanthus from waters off northern Argentina. Folia Parasitologica. 2013; 60(4):359–64. PMID: 24261137

61. Bui S, Dalvin S, Dempster T, Skulstad OF, Edvardsen RB, Wargelius A, et al. Susceptibility, behaviour, and retention of the parasitic salmon louse (Lepeophtheirus salmonis) differ with Atlantic salmon population origin. Journal of Fish Diseases. 2018; 41(3):431–42. https://doi.org/10.1111/jfd.12707 PMID: 28921589

62. Ugolivik MS, Skorping A, Mennerat A. Parasite fecundity decreases with increasing parasite load in the salmon louse Lepeophtheirus salmonis infecting Atlantic salmon Salmo salar. Journal of Fish Diseases. 2017; 40(5):671–8. https://doi.org/10.1111/jfd.12547 PMID: 27594545

63. Tang D, Uyeno D, Nagasawa K. Species of Taeniacanthus Sumpf, 1871 (Crustacea: Copepoda: Tae- niacanthidae) parasitic on boxfishes (Tetraodontiformes: Aracanidae and Ostraciidae) from the Indo-
West Pacific region, with descriptions of two new species. Systematic Parasitology. 2011; 80(2):141–57. https://doi.org/10.1007/s11230-011-9318-7 PMID: 21898203

64. Kim IH, Moon SY. Ten new species of parasitic cyclopoid copepods (Crustacea) belonging to the families Bomolochidae, Philichthyidae, and Taeniacanthidae from marine fishes in Korea. Ocean Science Journal. 2013; 48(4):361–98.

65. Yurakhno VM, Ovcharenko MO, Holzer AS, Sarabeev VL, Balbuena JA. Kudoa unicaulis n. sp. (Myxosporea: Kudoidae) a parasite of the Mediterranean mullets Liza ramada and L. aurata (Teleostei: Mugilidae). Parasitology Research. 2007; 101(6):1671–80. https://doi.org/10.1007/s00436-007-0711-8 PMID: 17846792

66. Diebakate C, Fall M, Faye N, Toguebaye BS. Unicaulis marquesi n. sp. (Myxosporea, Multivalvulida) parasite des branches de Polydactylus quadrililis (Cuvier, 1829) (Poisson, Polyinidae) des côtes sénégalaises (Afrique de l’Ouest). Parasite. 1999; 6:231–5. French https://doi.org/10.1051/parasite/1999063231 PMID: 10511971

67. Al-Jufaili SH, Freeman MA, Machkevski VK, Al-Nabhani A, Palm HW. Morphological, ultrastructural, and molecular description of Unicaulis fatimae n. sp. (Myxosporea: Trilosporidae) of whitespotted rabbitfish (Siganus canaliculatus) in Omani waters. Parasitology Research. 2016; 115(3):1173–84. https://doi.org/10.1007/s00436-015-4851-y PMID: 26693719

68. Petter A, Lèbre C, Radujković B. Nematodes parasites de poissons Osteichthyes de l’Adriatique Méditerranéen. Acta Adriatica. 1984; 25(1/2):205–21. French

69. Bartlett CM. Morphogenesis of Contraecaeum rudolphii (Nematoda: Ascaridoidea), a parasite of fish-eating birds, in its copepod precursor and fish intermediate hosts. Parasite. 1996; 3(4):367–76.

70. Tavakol S, Smit WJ, Smit WJ. Distribution of Contraecaeum (Nematoda: Anisakidae) larvae in freshwater fish from the Northern Regions of South Africa. African Zoology. 2015; 50(2):133–9.

71. Moravec F, Justine J Lou. Two new species of nematode parasites, Cucullanus epinepheli sp. n. (Cucullanidae) and Procamallanus (Spirocamallanus) sinespinis sp. n. (Camallanidae), from marine serrani and haemulid fishes offNew Caledonia. Folia Parasitologica. 2017; 64(1):011.

72. Moravec F, Fiala I, Dyková I. New data on the morphology of Dichelyne hartwichi (Nematoda, Cucullanidae), a parasite of freshwater tetraodontid fishes (Tetraodon spp.) in Thailand. Acta Parasitologica. 2011; 56(4):433–7

73. Valtonen ET, Valtonen T. Cystidicol a farionis as a swimblander parasite of the whitefish in the Bothnian Bay. Journal of Fish Biology. 1978; 13(5):557–61.

74. Muzzall PM. Parasites of Pacific Salmon, Oncorhynchus spp., from the Great Lakes. Journal of Great Lakes Research. 1995; 21(2):248–56.

75. Navone GT, Sardella NH, Timi JT. Larvae and adults of Hysterothylicium aduncum (Rudolphi, 1802) (Nematoda: Anisakidae) in fishes and crustaceans in the south west Atlantic. Parasite. 1998; 5(2):127–36. https://doi.org/10.1051/parasite/1998052127 PMID: 9754308

76. Özer A, Kornyychu YM, Yurakhno VM, Öztürk T. Seasonality and host-parasite interrelationship of Hysterothylicium aduncum (Nematoda) in whiting Merlangius merlangus off the southern and northern coasts of the Black Sea. Helminthology. 2016; 53(3):248–56.

77. Cross JH, Basaca-Sevilla V. Capillariosis philippinensis: a fish-borne parasitic zoonosis. The Southeast Asian journal of tropical medicine and public health. 1991; 22 Suppl(2):153–7.

78. Moravec F, Justine J-L. Capillaria plec tropomri n. sp. (Nematoda: Capillaridae), a new intestinal parasite of the leopard coral grouper Plectropomus leopardus (Serranidae) off New Caledonia. Parasite. 2014; 21:76. https://doi.org/10.1051/parasite/2014076 PMID: 25531932

79. Neofar L, Euzet L, Olivier G. Lamellodiscus (Plathelminthes, Monogenea, Diplectanidae) nouveaux parasites branchiaux des poissons marins du genre Pagrus (Teleostei, Sparidae). Zoosystema. 2004; 26(3):365–76. French

80. Noisy D, Maillard C. Microhabitat branchial préférentiel de Microcote y chrysophi ri (Monogenea: Microcotyleidae) in Pagrus (Teleostei, Sparidae). Annales de Parasitologie Humaine et Comparée. 1980; 33–40. French

81. Ayadi ZEM, Gey D, Justine J Lou, Tazeroufi F. A new species of Microcote (Monogenea: Microcotylei dae) from Scopraena notata (Teleostei: Scorpaenidae) in the Mediterranean Sea. Parasitology International. 2017; 66(2):37–42. https://doi.org/10.1016/j.parint.2016.11.004 PMID: 27840197

82. Pantermak Z, Diamant A, Abelson A. Co-invasion of a Red Sea fish and its ectoparasitic monogenean, Polylabris cf. mamaevi into the Mediterranean: Observations on oncomiracidium behavior and infection levels in both seas. Parasitology Research. 2007; 100(4):721–7. https://doi.org/10.1007/s00436-006-0330-9 PMID: 17096147
83. Bayoumy EM, El-Lamie MMM, Derwa HIM. First Report of Polylabris lingaoensis (Monogenoidea: Polypisthocotylea) Infesting the Gillss of Acanthopagrus bifasciatus From the Red Sea, Off. World Journal of Fish and Marine Sciences. 2015; 7(3):209–13.

84. Timi JT, Martorelli SR, Sardella NH. Digeneric trematodes parasitic on Engraulis anchoita (Pisces: Engraulidae) from Argentina and Uruguay. Folia Parasitologica. 1999; 46(2):132–8. PMID: 10425743

85. Born-Torrijos A, Poulin R, Pérez-del-Olmo A, Cülurgioni J, Raga JA, Holzer AS. An optimised multi-host trematode life cycle: fish discards enhance trophic parasite transmission to scavenging birds. International Journal for Parasitology. 2016; 46(11):745–53. https://doi.org/10.1016/j.ijpara.2016.06.005 PMID: 27492876

86. Alama-Bermejo G, Montero FE, Raga JA, Holzer AS. Skoulekia meningialis n. gen., n. sp. (Digenea: Apoecotylidae Odhner, 1912) a parasite surrounding the brain of the Mediterranean common two-banded seabream Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (Teleostei: Sparidae): Description, molecular phylogeny, habitat and pathology. Parasitology International. 2011; 60(1):34–44. https://doi.org/10.1016/j.parint.2010.10.001 PMID: 20950706

87. Palacios-Abella JF, Georgieva S, Mele S, Raga JA, Isbert W, Kostadinova A, et al. Skoulekia erythrina n. sp. (Digenea: Apoecotylidae): a parasite of Pagellus erythrinus (L.) (Periconomorph: Sparidae): from the western Mediterranean with an amendment of the generic diagnosis. Systematic Parasitology. 2017; 94(6):669–88. https://doi.org/10.1007/s11230-017-9733-5 PMID: 28573546

88. Ahuir-Baraja AE, Padros F, Palacios-Abella JF, Raga JA, Montero FE. Accacoelium contortum (Trematoda: Accacoelidiidae) a trematode living as a monogenean: Morphological and pathological implications. Parasites and Vectors. 2015; 8(1):540

89. Bartoli P, Bray RA. Two species of the fish digenean genus Rhipidocotyle Diesing, 1858 (Bucephalidae) reported for the first time from European seas. Systematic Parasitology. 2005; 62(1):47–58. https://doi.org/10.1007/s11230-005-3170-6 PMID: 16132870

90. Al-Zubaidy AB. First record of Lecithochirium sp. (Digenea: Hemiuriidae) in the marine fish Carangoides bajad from the Red Sea, Coast of Yemen. Journal of King Abdulaziz University, Marine Science. 2010; 21(1):85–94.

91. Morsy K, Bashtar AR, Abdel-Ghafar F, Baksh W. First record of Lecithochirium grandiporum (Digenea: Hemiuriidae) infecting the lizard fish Saurida tumbil from the Red Sea. Parasitology Research. 2012; 111(6):2339–44. https://doi.org/10.1007/s00436-012-3111-7 PMID: 22968948

92. Ndiaye PI, Quilichini Y, Seme A, Tkach V V, Ba CT, Marchand B. Ultrastructural characters of the spermatozoa in Digeneans of the genus Lecithochirium Lühe, 1901 (Digenea, Hemiuriidae), parasites of fishes: comparative study of L. microstomum and L. musculus. Parasite. 2014; 21:49. https://doi.org/10.1051/parasite/2014050 PMID: 25275216

93. Sripa B, Bethony JM, Siltihaworn P, Kaewkes S, Maijang E, Loukas A, et al. Opisthorchiasis-associated cholangiocarcinoma in Thailand and Laos. Acta Tropica. 2011; 120 Suppl 1 (Suppl 1):S158–68.

94. Radujkovic B, Sundic D. Parasic flatworms (Platyhelminthes: Monogenea, Digenea, Cestoda) of fishes from the Adriatic Sea. Natura Montenegro. 2014; 13(1):7–280.

95. Muñoz G, Díaz PE. Checklist of parasites of labrid fishes (Pisces: Labridae). 2015. 95 p.

96. Lainson R. Theileria electrophori n.sp., a parasite of the electric eel Electrophorus electricus (Osteichthyes: Cypriniformes: Gymnotidae) from Amazonian Brazil. Memorias do Instituto Oswaldo Cruz. 2007; 102(2):155–7 https://doi.org/10.1590/s0074-02762007005000004 PMID: 17426878

97. Baker JR, Muller R, Rollinson D. Advances in parasitology. Volume 36. Academic Press; 1995. 415 p.

98. Alvarez-Pellitero P, Sijá-Bobadilla A. Cryptosporidium molnari n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, Sparus aurata L. and Dicentrarchus labrax L. International Journal for Parasitology. 2002; 32(6):1007–21. https://doi.org/10.1016/s0020-7519(02)00058-9 PMID: 12076630

99. Ryan U. Cryptosporidium in birds, fish and amphibians. Experimental Parasitology. 2010; 124(1):113–20. https://doi.org/10.1016/j.exppara.2009.02.002 PMID: 19545515

100. Diouf JN, Togouebye B. Eimeria sparri n. sp. (Apicomples, Eimeridae) parasite of Sperus caeruleos-ticuus (Valenciennes, 1830), (Pisces, Sparidae) from the coast of Senegal. Parasite. 1996; 3(4):351–5.

101. Gjurcevic E, Kuzir S, Bazdaric B, Matanovic K, Debelic I, Marino F, et al. New data on Eimeria dicer- trachis (Apicomplexa: Eimeridae), a common parasite of farmed European sea bass (Dicentrarchus labrax) from the mid-eastern Adriatic. Veterinarski Arhiv. 2017; 87(1):77–86.

102. Alvarez-Pellitero P, M. C., Gonzalez-Lanza M. Goussia carpell (Protozoa, Apicomplexa) in cyprinid fish of the Duero basin (NW Spain). Aspects of host-parasite relationships. Journal of Applied Ichthyology. 1986; 2(3):125–30.
103. Lovy J, Friend SE. Intestinal coccidiosis of anadromous and landlocked alewives, *Alosa pseudoharengus*, caused by *Goussia ameliana* n. sp. and *G. alosi* n. sp. (Apicomplexa: Eimeriidae). International Journal for Parasitology: Parasites and Wildlife. 2015; 4(2):159–70. https://doi.org/10.1016/j.ijppaw.2015.02.003 PMID: 25853050

104. Nilsen F. Description of *Trichodina hippoglossi* n. sp. from farmed Atlantic halibut larvae *Hippoglossus hippoglossus*. Diseases of Aquatic Organisms. 1995; 21:209–14.

105. Kritsky DC, Heckmann R. Species of *Dactylogyrus* (Monogeneidea: Dactylogyridae) and *Trichodina mutabilis* (Ciliata) Infesting Koi Carp, *Cyprinus carpio*, During Mass Mortality at a Commercial Rearing Facility in Utah, U.S.A. Comparative Parasitology. 2002; 69(2):217–8.

106. Guillon L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, Massana R, et al. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environmental Microbiology. 2008; 10(12):3349–65. https://doi.org/10.1111/j.1462-2920.2008.01731.x PMID: 18771501

107. Budischak SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO. A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Molecular Ecology Resources. 2015; 15(5):1112–9. https://doi.org/10.1111/1755-0998.12382 PMID: 25644900

108. Bittleston LS, Baker CCM, Strominger LB, Pringle A, Pierce NE. Metabarcoding as a tool for investigating arthropod diversity in *Nepenthes* pitcher plants. Austral Ecology. 2016; 41(2):120–32.

109. Avramenko RW, Redman EM, Lewis R, Bichuette MA, Palmeira BM, Yazwinski TA, et al. The use of nemabiole metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. International Journal for Parasitology. 2017 Nov 1; 47(13):893–902. https://doi.org/10.1016/j.ijpara.2017.06.006 PMID: 28797791

110. Wilcox JJS, Hollocher H. Unprecedented Symbiont Eukaryote Diversity Is Governed by Internal Trophic Webs in a Wild Non-Human Primate. Protist. 2018; 169(3):307–20 https://doi.org/10.1016/j.protis.2018.03.001 PMID: 29803114

111. Bass D, Stentiford GD, Littlewood DTJ, Hartikainen H. Diverse Applications of Environmental DNA Methods in Parasitology. Trends in Parasitology. 2015; 31(10):499–513. https://doi.org/10.1016/j.pt.2015.06.013 PMID: 26433253

112. Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN. Quantifying sequence proportions in a DNA-based diet study using ion Torrent amplicon sequencing: Which counts count? Molecular Ecology Resources. 2013; 13(4):620–33. https://doi.org/10.1111/1755-0998.12103 PMID: 23590207

113. Jorge F, Carretero MA, Roca V, Poulin R, Perera A. What you get is what they have? Detectability of intestinal parasites in reptiles using faeces. Parasitology Research. 2013; 112(12):4001–7. https://doi.org/10.1007/s00436-013-3588-8 PMID: 23999900

114. Gillespie TR. Noninvasive Assessment of Gastrointestinal Parasite Infections in Free-Ranging Primates. International Journal of Primatology. 2008; 27(4):1129–43

115. Fouhy F, Clooney AG, Stanton C, Claesson MJ, Cotter PD. 16S rRNA gene sequencing of mock microbial populations- impact of DNA extraction method, primer choice and sequencing platform. BMC Microbiology. 2016 Dec 24; 16(1):123. https://doi.org/10.1186/s12866-016-0738-z PMID: 27342980

116. Thomas AC, Deagle BE, Eveson JP, Harsch CH, Trites AW. Quantitative DNA metabarcoding: Improved estimates of species proportional biomass using correction factors derived from control material. Molecular Ecology Resources. 2016; 16(3):714–26. https://doi.org/10.1111/1755-0998.12490 PMID: 26602877

117. Santamaria M, Fosso B, Consiglio A, De caro G, Grillo G, Licciulli F, et al. Reference databases for taxonomic assignment in metagenomics. Briefings in Bioinformatics. 2012; 13(6):682–95. https://doi.org/10.1093/bib/bbs036 PMID: 22786794

118. Gibbons FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, et al. 25 years of serving the community with ribosomal RNA gene reference databases and tools. Journal of Biotechnology. 2017; 261:169–76. https://doi.org/10.1016/j.jbiotec.2017.06.1198 PMID: 28648396

119. Bortolus A. Error Cascades in the Biological Sciences: The Unwanted Consequences of Using Bad Taxonomy in Ecology. Ambio. 2008; 37(2):114–9. https://doi.org/10.1579/0044-7447(2008)37[114:ectbe]2.0.co;2 PMID: 18488554

120. Kvist S. Barcoding in the dark?: A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. Molecular Phylogenetics and Evolution. 2013; 69(1):39–45. https://doi.org/10.1016/j.ympev.2013.05.012 PMID: 23721749

121. Tinsley RC, Owen RW. Studies on Biology of *Protopoly stoma xenopodis* (Monogeneidea)–the oncomiracidium and life-cycle. Parasitology. 1975; 71(3):445–63.
122. Buchmann K. Some histochemical characteristics of the mucous microenvironment in four salmonids with different susceptibilities to gyrodactylid infections. Journal of Helminthology. 1998; 72(2):101–7.

123. Whittington ID, Cribb BW, Hamwood TE, Halliday JA. Host-specificity of monogenean (platyhelminth) parasites: A role for anterior adhesive areas? International Journal for Parasitology. 2000; 30(3):305–20. https://doi.org/10.1016/s0020-7519(00)00066-0 PMID: 10719124

124. Kearns GC. Experiments in host-finding and host-specificity in the monogenean skin parasite Entobdella solaea. Parasitology. 1967; 57(3):585–605. https://doi.org/10.1017/s00311820000072450 PMID: 6069119

125. Buchmann K, Lindenstrøm T. Interactions between monogenean parasites and their fish hosts. International Journal for Parasitology. 2002; 32(3):309–19. https://doi.org/10.1016/s0020-7519(01)00332-0 PMID: 11835971

126. Galaktionov K, Malkova II, Irwin SWB, Saville H, Maguire JG. The structure and formation of metacercarial cysts in the trematode family Microphallidae Travassos 1920. Journal of helminthology. 1997; 71(1):13–20. PMID: 9166434

127. Poulin R, Cribb TH. Trematode life cycles: short is sweet? Trends in Parasitology. 2002 Apr 1; 18(4):176–83 PMID: 11998706

128. Harder W. Anatomy of fishes. Schweizerbart; 1975. 612 p.

129. Li L, Ali AH, Zhao WT, Lü L, Xu Z. First report on nematode parasite infection in the yellowbar angelfish Pomacanthus maculosus (Perciformes: Pomacanthidae) from the Iraqi coral reef, with description of a new species of Cucullanus (Nematoda: Ascaridida) using the integrated approaches. Parasitology International. 2016; 65(6):677–84.

130. Smith JD, Lankester MW. Development of swim bladder nematodes (Cystidicola spp.) in their intermediate hosts. Canadian Journal of Zoology. 1979; 57(9):1736–44. PMID: 540279

131. Black GA, Lankester MW. Migration and development of swim-bladder nematodes, Cystidicola spp. (Habronematoida), in their definitive hosts. Canadian journal of Zoology. 1980; 58(11):1997–2005.

132. Koie M. The life cycle of Dichelyne (Cucullanellus) minutus (Nematoda: Cucullanidae). Folia Parasitologica. 2001; 48(4):304–10. PMID: 11817453

133. Koie M. The life-cycle of the flatfish nematode Cucullanus heterochrous. Journal of helminthology. 2001; 74(4):323–8.

134. Veciana M, Chaisiri K, Morand S, Ribas A. Aonchotheca yannickchavali n. sp. (Nematoda: Capillariidae) in Bandicota indica (Bechstein, 1800) and Bandicota savilei (Thomas, 1916) (Rodentia: Muridae) collected from Thailand. Agriculture and Natural Resources. 2016; 50(6):470–3.