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NOTE

Moorea BIOCODE barcode library as a tool for understanding predator–prey interactions: insights into the diet of common predatory coral reef fishes

M. Leray · J. T. Boehm · S. C. Mills · C. P. Meyer

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Abstract Identifying species involved in consumer–resource interactions is one of the main limitations in the construction of food webs. DNA barcoding of prey items in predator guts provides a valuable tool for characterizing trophic interactions, but the method relies on the availability of reference sequences to which prey sequences can be matched. In this study, we demonstrate that the COI sequence library of the Moorea BIOCODE project, an ecosystem-level barcode initiative, enables the identification of a large proportion of semi-digested fish, crustacean and mollusks found in the guts of three Hawkfish and two Squirrelfish species. While most prey remains lacked diagnostic morphological characters, 94% of the prey found in 67 fishes had >98% sequence similarity with BIOCODE reference sequences. Using this species-level prey identification, we demonstrate how DNA barcoding can provide insights into resource partitioning, predator feeding behaviors and the consequences of predation on ecosystem function.

Keywords Trophic interactions · Diet analysis · Food web · DNA identification · Hawkfish · Squirrelfish

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M. Leray (✉) · S. C. Mills
Laboratoire d'Excellence "Corail" USR 3278 CNRS EPHE, CRIOBE-CBETM, Université de Perpignan, 56 Avenue Paul Alduy, 66860 Perpignan Cedex, France
e-mail: leray.upmc@gmail.com

M. Leray · C. P. Meyer
Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC-163, Washington, DC 20013, USA

J. T. Boehm
Biology Department, Queens College, City University of New York, Flushing, NY 11367, USA

J. T. Boehm
The Graduate Center, City University of New York, New York, NY 10016, USA

Introduction

The high biodiversity of coral reefs means that ecologists are confronted with a complex task of species identification in their quest for understanding community-level processes and interactions. Subtle differences in diagnostic phenotypic characters, presence of morphologically cryptic and undescribed species, and lack of identification guides for early life stages hinder reliable species-level identification in routine ecological studies (Hebert et al. 2003). Fortunately, DNA barcoding can be used to supplement traditional taxonomy when their DNA matches species-specific sequences available in barcode reference libraries.

Witnessing direct predator–prey interactions in the field is challenging (Merfield et al. 2004); therefore, DNA-based techniques are increasingly used for characterizing predator diet from feces/gut content (King et al. 2008). Prey-specific DNA fragments can be amplified from semi-digested prey (Zaidi et al. 1999; Dunn et al. 2010), and prey sequences can be identified if reference barcode databases contain a comprehensive list of species consumed. Despite the growing availability of reference databases, large proportions remain unidentified particularly from generalist diets (Blankenship and Yayanos 2005; Dunn et al. 2010).

Among various ongoing barcoding initiatives, the Moorea BIOCODE project (http://www.mooreabiocode.org) is an "All Taxa Biotic Inventory" whose goal is to provide

a library of genetic markers for all non-microbial species of the French Polynesian tropical ecosystem. From 2006 to 2010, teams of researchers have worked to sample macrobiotic species (>5,670 species ≥2 mm) of which 3,877 (68%) are coral reef species. All specimens were identified morphologically to lowest taxon level, photographed and their tissue sampled for DNA barcoding. A library of species-specific DNA signatures amplified from a single homologous region, the cytochrome *c* oxidase subunit I, was constructed for most animals. Reference specimens were sent to museum collections. All information, from the collection of specimens to their sequencing, was centralized in BIOCODE's field and laboratory information management systems. As of April 2011, reference data exist for 28 marine phyla, with an emphasis on arthropods, chordates and mollusks (http://biocode.berkeley.edu). The barcode inventory of this model ecosystem will allow researchers to overcome many limitations inherent in morphology-based identification when species-level information is required, for example to understand predator feeding ecology and food web dynamics.

Here, we used direct sequencing to identify prey remains in the stomachs of five common predator fish on Moorean reefs with contrasting feeding regimes: three hawkfish, *Paracirrhites arcatus*, *P. forsteri* and *P. hemistictus* (Order: Perciformes; Family: Cirrhitidae), and two squirrelfish, *Sargocentron microstoma* and *S. tiere* (Order: Beryciformes; Family: Holocentridae). The three hawkfish species commonly occupy coral colonies of the genus *Pocillopora* where they sit and wait for prey during the day (Kane et al. 2009). In contrast, the two squirrelfish species actively look for benthic prey at night (Arias-Gonzalez et al. 1998; Randall 2005). We aimed to: (1) assess the proportion of prey that matched BIOCODE reference sequences in order to evaluate the efficacy of BIOCODE's efforts to inventory macro-invertebrates and fishes and (2) investigate how species-level prey identification could provide insights into resource partitioning and feeding behaviors in relation to the life history traits of predators as well as into the consequences of predation on ecosystem function. As this is the first attempt to characterize the diet of coral reef-associated predators using DNA-based techniques, this approach provides great promise for understanding complex trophic interactions.

Materials and methods

Fish collection and gut content dissection

A total of 67 adult carnivorous fish (33 *P. arcatus*, 11 *P. forsteri*, 7 *P. hemistictus*, 8 *S. microstoma* and 8 *S. tiere*) were speared on the north shore forereef of Moorea, French Polynesia (17°30′S, 149°50′W), during the Austral Winters of 2009 and 2010. The diurnal species (*Paracirrrhites* spp.) were sampled at dusk, while the nocturnal predators (*Sargocentron* spp.) were collected both at dawn and 2 hrs after dusk. We did not observe prey regurgitation while capturing predators. Fishes were preserved in cold 50% ethanol in situ. In the laboratory, stomach contents were dissected, and all visually distinguishable prey items identified to the lowest taxon possible based on morphology. Tissue samples were then taken from these prey remains, rinsed with distilled water, counted and placed in individual tubes for extraction and barcoding. Remaining stomach contents were discarded, which is likely to have biased our results toward larger and hard prey items that better resist digestion.

DNA analysis and sequence identification

Total genomic DNA was extracted using automated phenol–chloroform extraction with the Autogenprep 965 (Autogen, MA) with a final elution volume of 100 µl. COI fragments were PCRed as 20-µl reactions with 0.6 µl of 10 µM of each universal forward and reverse primers (Folmer et al. 1994), 0.2 µl of Biolase *taq* polymerase (Bioline) 5 U μl^{-1}, 0.8 µl of 50 mM Mg^{2+}, 1 µl of 10 µM dNTP and 1 µl of genomic DNA. PCR conditions were as follows: 5 min at 95°C; 35 cycles of 30 s at 95°C; 30 s at 48°C; 45 s at 72°C; and a final 5 min at 72°C. Sequences were identified based on similarity to the BIOCODE sequence library using BLAST (Altschul et al. 1997) searches performed in Geneious Pro 5.0.3 (Biomatters). COI sequences were then assigned to taxonomic groups according to criteria defined by Machida et al. (2009) and Plaisance et al. (2009). Sequences were considered to match reference specimens when sequence similarity was >98%. In order to test whether our sampling effort was sufficient, expected species accumulation curves with 95% confidence intervals were computed using EstimateS (Colwell et al. 2004).

Results and discussion

Of the 67 fish speared, 52 had visually distinguishable prey remains in their stomach encompassing 102 total individual prey items. The majority of fish had only one visually distinguishable prey item, but number of prey items ranged from 0 to 7 (Fig. 1). Based on the size of observed hard parts, all crustacean and mollusks were consumed as adults while fish had been preyed upon as juveniles. Morphological identification of crustacean appendages (62 items) and fish fins (23 items) rarely provided identifications lower than the family level. Only two crustacean prey

items from two *P. arcatus* were identified to the species level (*Menaethius monoceros)*. Morphological identification therefore achieved less than 2% success at species level, later verified by DNA.

On the other hand, COI sequences obtained from 96 (of 102) prey items showed higher than 98% levels of sequence similarity with BIOCODE reference sequences (Table 1). In comparison, only 16 prey items (8 species) had less than 98% similarity with sequences in GenBank (excluding BIOCODE-generated sequences—Table 1). Of the four remaining crustacean sequences without >98% matches, one was identified to the family Parthenopidae (85% similarity), while three remaining crustaceans could not be confidently assigned to any taxonomic group (<80% similarity). Two sequences matching bacterial DNA fragments were discarded. Overall, 94% of the sequences were identified to species level using COI sequences, demonstrating the efficiency with which BIOCODE has sampled fish, macro-crustaceans and macro-mollusks from the Moorea reef community (GenBank accession numbers JN107891–JN107990).

Despite the high level (>98%) of sequence similarity, BIOCODE reference specimens could not provide species names to 13 prey items: 1 fish, 10 crustacean and 2 mollusks. These vouchered specimens either are undescribed species (e.g., Galatheidae) or require further taxonomic work for correct identification. Two prey items matched specimens with identical names (*Chlorodiella laevissima*) but are genetically distinct (K2P dist. $\pm$ SE = 0.103 $\pm$ 0.014), suggesting the presence of cryptic lineages. Taxonomic refinement and new species descriptions are ongoing as part of the BIOCODE project. A large proportion of the crustacean database is publically available on BOLD (Project MBMIA), and fishes will be released upon final acceptance of a manuscript under revision. All comparative data will be made public by July 2012 according to funding obligations.

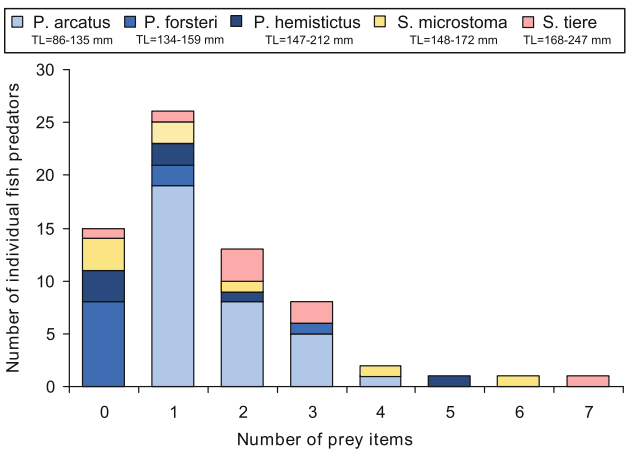

Fig. 1 Number of prey items from the guts of 67 fishes belonging to five predator species. *TL* total length

The rarefaction curve indicates that additional fish collections would be required to better characterize the diet of these predatory species (Fig. 2). Additionally, despite these five species being known to consume mostly other fish, crustacean and mollusks, our dietary analysis likely missed soft-bodied prey species that can be detected using PCR amplification and sequencing from the whole-gut content tissue homogenate (Jarman et al. 2004; Deagle et al. 2009). Despite the need for additional sampling of predator diet, we discuss how prey identification to the species level using barcoding can provide novel insights into resource partitioning, predator feeding behaviors and the potential consequences of predation on ecosystem function.

Firstly, the degree to which specialization on food resources enables species coexistence and shapes community structure has long been debated (Jones 1991). This debate has been limited as estimates of the degree of diet overlap between predator species greatly depends on taxonomic resolution achieved in dietary studies (Longenecker 2007). *Paracirrhites forsteri* and *P. hemistictus*, both large diurnal ambush piscivorous species commonly found among the branches of large pocilloporid corals (Kane et al. 2009), had narrow and largely overlapping diets with a majority of *Chromis vanderbilti* (80 and 67% of prey items, respectively) in their guts (Table 1; Fig. 3). Alternatively, *S. microstoma* and *S. tiere*, both mobile active nocturnal feeders (Arias-Gonzalez et al. 2004), have broad diets and did not have a single prey species in common (Fig. 3). Their different diets reinforce the value of species-level prey identifications, as familial level would have failed to elucidate the true trophic structure of these sister species. *Paracirrhites arcatus* only shared one single prey species with its congenerics, whereas eight prey species were shared with predators that differ in microhabitat use and time of feeding activity (Fig. 3). These results, which must be treated with caution due to the limited number of samples and potentially different digestion rates, suggest that timing of activity, habitat partitioning and hunting mode may not accurately predict resource partitioning among reef fish species. The barcode inventory of the Moorea ecosystem provides an ideal testing ground for further exploration of the role that resource specialization plays in shaping patterns of biodiversity.

Secondly, our findings indicate that a few prey species may provide a considerable source of energy to predators in the Moorea food web. For instance, *C. vanderbilti* was commonly consumed by *P. forsteri* and *P. hemistictus* and *Liocarpilodes integerrimus* by *P. arcatus* and *S. microstoma* (Table 1), suggesting that they may be preferential targets or highly abundant on Moorean reefs. Empirical evidence suggests that generalist piscivorous predators forage non-selectively and consume prey in proportion to their abundance (Heinlein et al. 2010—study in Moorea).

Table 1 Summary of prey items successfully identified from the stomach contents of fish using COI amplification and BLAST searches in the BIOCODE barcode library

Subphylum/class	Prey ID	% identity BIOCODE	*n*	BIOCODE specimen	Predator ID
Actinopterygii	*Chromis acares*	99	1	MParis0005	ST
	Chromis iomelas	99.8	2	MParis0055	PH
	Chromis vanderbilti	99–99.5	12	MParis0195	PA, PF, PH
	Cirripectes variolosus	100	1	MParis0213	PA
	Eviota disrupta	99.7	1	MParis0174	PA
	Eviota sp.	99.4	1	XMOO-0047	PA
	Neocirrhites armatus[a]	99.2	2	MParis0007	PF, PH
	Pseudogramma polyacanthum	99.5	1	MParis0012	ST
	Synodus binotatus[a]	98.8	1	MParis586	SM
	Valenciennea strigata[a]	100	1	MParis0906	SM
Crustacea	*Acanthanas pusillus*	99.1	1	BMOO-02811	ST
	Alpheus dolerus	99.7–100	2	BMOO-00430	PA
	Aniculus retipes[a]	100	1	BMOO-01743	ST
	Axiidae	98.2	1	BMOO-01079	PA
	Brachycarpus biunguiculatus	98	1	BMOO-05348	PA
	Calappa gallus	100	1	BMOO-02116	PA
	Chlorodiella barbata	100	2	BMOO-00324	PA, SM
	Chlorodiella crispipleopa	99.5–99.7	3	BMOO-00726	PA
	Chlorodiella laevissima	98.4	1	BMOO-01191	SM
	Chlorodiella laevissima	99.8	3	BMOO-02899	ST
	Cyclodius ungulatus	98–99.8	2	BMOO-01049	PA, SM
	Daldorfia sp.	99.2	1	BMOO-05257	PA
	Epialtidae	99.2	1	BMOO-03230	PA
	Etisus frontalis	99.7	1	BMOO-00194	PA
	Galathea mauritiana[a]	99.5–99.8	2	XMOO-0011	PA, SM
	Galathea sp.[a]	99.5–99.8	4	BMOO-02353	PA, ST
	Gnathiidae	98.5	1	No voucher	PA
	Gonodactylus affinis	99.4	1	jg8	PA
	Huenia sp.	99.2	1	BMOO-03531	PA
	Liocarpilodes integerrimus	98.8–99.8	7	BMOO-01576	PA, SM
	Medaeus elegans	99.4	1	BMOO-04008	PA
	Menaethius monoceros	98.5–99.5	2	BMOO-03072	PA
	Menaethius orientalis	99	1	BMOO-01847	ST
	Metalpheus nanus	99.7	1	BMOO-02919	PA
	Palaemonella rotumana	100	1	BMOO-02250	PA
	Palmyria palmyrensis	100	1	BMOO-01358	PA
	Parthenopidae	85	1	No match	ST
	Perinia tumida	98.5	1	XMOO-0383	PA
	Petrolisthes sp.	98.7%	1	BMOO-02308	PA
	Phylladiorhynchus sp.	99.6	1	BMOO-03262	PA
	Phylladiorhynchus integrirostris	99.2–99.7	4	BMOO-01856	PA
	Pilodius flavus	99.5–100	4	BMOO-02998	PA, ST
	Pilodius pugil	100	2	BMOO-01102	SM
	Saron marmoratus	99	1	BMOO-02912	ST
	Saron sp.	98.3	1	No voucher	PA
	Thalamita sp.	98	2	BMOO-05357	PA, SM
	Trapezia flavopunctata	99.7	1	BMOO-02830	ST
	Trapezia tigrina	100	2	XMOO-0202	PA

Table 1 continued

Subphylum/class	Prey ID	% identity BIOCODE	*n*	BIOCODE specimen	Predator ID
	Xanthias latifrons	99.5	1	BMOO-04066	ST
Mollusk	*Deniatys dentifer*[a]	98	3	BMOO-02659	SM
	Erato sp.[a]	99.2	2	BMOO-02545	ST
	Julia zebra	98.1	1	BMOO-03193	PA
	Stomatella rosaceus	99.4	1	BMOO-01483	ST
	Stomatolina sp.	99.5	1	BMOO-06132	ST

The BIOCODE reference specimen ID is reported for each prey so that photographs and additional information can be obtained at http://biocode.berkeley.edu

n = number of prey. PA: *Paracirrhites arcatus*; PF: *Paracirrhites forsteri*; PH: *Paracirrhites hemisticus*; ST: *Sargocentron tiere*; SM: *Sargocentron microstoma*

[a] Indicates prey COI sequences that also had >98% similarity with sequences in GenBank

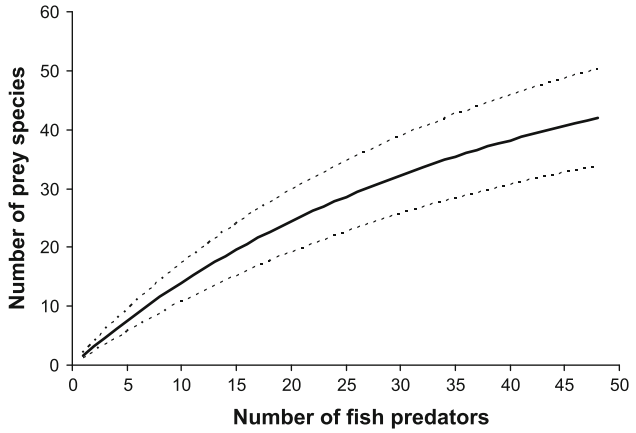

Fig. 2 Rarefaction curve for number of prey species as a function of fish collected with prey in their stomach ($N = 52$). *Dashed lines* represent 95% CI

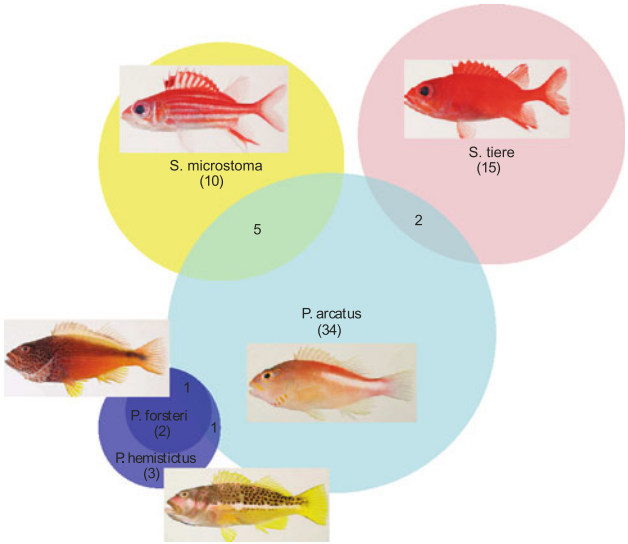

Fig. 3 Venn diagram illustrating the overlap of prey species consumed by predator fish species. *Circle* sizes are proportional to total number of prey species consumed by each predator (*parentheses*), and *overlap area* between *circles* is proportional to number of shared prey species. Photographs were provided by Jeffrey Williams

Conversely, Longenecker (2007) observed a different pattern for predators; despite large ephemeral increases in the abundance of certain invertebrate prey species, they were not increasingly consumed by predators. Invertebrate population sizes, as well as temporal and spatial patterns of variation in abundance, remain unknown in Moorea. Therefore, further studies should be conducted to evaluate diet selection and the role keystone prey species (Power et al. 1996) play in the persistence of coral reef predators.

Finally, DNA barcoding revealed that the fish predators feed on prey which themselves are important for habitat maintenance and ecosystem functioning. *Paracirrhites forsteri* and *P. hemistictus* consumed *Neocirrhites armatus*, and *P. arcatus* had fed upon *Trapezia flavopunctata* and *T. tigrina*, which are all known to benefit Pocilloporids. Resident fish such as *N. armatus* provide nutrients to host polyps (Holbrook et al. 2008), while *Trapezia* species increase the survival and growth of their host by removing sediment from coral tissue (Stewart et al. 2006), defending against corallivorous seastars (Glynn 1983) and removing parasitic vermetid gastropod nets (Stier et al. 2010). Further investigation should determine the functional consequences resulting from the predation pressure highlighted in this study.

Overall, we show that the quality of the barcode reference database in Moorea will enable researchers to uncover the complexity and spatial–temporal dynamics of food webs not just in French Polynesia but also throughout the Western Pacific where taxon ranges likely extend. DNA barcoding removes subjectivity biasing prey identification compared to visual identification and is particularly valuable for reef fish prey identification given the high ecosystem biodiversity. Such promises for understanding the functioning of natural systems should encourage further ecosystem-based barcoding initiatives worldwide.

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