First genetically confirmed record of the invasive devil firefish  

*Pterois miles* (Bennett, 1828) in the Mexican Caribbean

Irán A. Guzmán-Méndez¹, Renata Rivera-Madrid², Pindaro Díaz-Jaimes³, María del C. García-Rivas⁴, Margarita Aguilar-Espinosa¹ and Jesús E. Arias-González¹ *  

¹Laboratorio de Ecología de Ecosistemas de Arrecifes Coralinos, Departamento de Recursos del Mar, Centro de Investigación y de Estudios Avanzados del I.P.N.- Unidad Mérida, A.P. 73, Cordemex, 97310 Mérida, Yucatán, México  
²Centro de Investigación Científica de Yucatán, A.C. Calle 43 No. 130, Col. Chuburná de Hidalgo, C.P. 97205 Mérida, Yucatán, México  
³Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Apartado Postal 70-305, México D.F. 04510, México  
⁴Comision Nacional de Áreas Naturales Protegidas, Parque Nacional Arrecifes de Puerto Morelos. Av. Javier Rojo Gómez, Mz. 8 Lote 4, C.P. 77580, Puerto Morelos, Quintana Roo, México  

*Corresponding author  
E-mail: earias@cinvestav.mx  

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**Abstract**

Devil firefish *Pterois miles* (Bennett, 1828) is a species native to the Indo-Pacific, described by Bennett in 1828 (Schultz 1986). Historically, there have been several discussions over the taxonomic status of *Pterois volitans* (Linnaeus, 1758) and *Pterois miles* (Smith 1957; Beaufort and Briggs 1962; Randall 1983). However, the most recent taxonomic treatment defines them as separate species based on statistical analysis of meristic and morphometric characters (Schultz 1986). A genetic analysis revealed that these two species diverged from a common ancestor 2.4 to 8.3 million years ago, and the separation of their lineages is relatively recent (Kochzius et al. 2003). Morris et al. (2011) observed that both species have similar morphological characteristics, life cycles, habits, and dispersal potential. These similarities may cause confusion in the identification of the two species and hinder population studies. Molecular studies based on the use of mitochondrial DNA are very useful in addressing species’ identification (Freshwater et al. 2009a; Hamner et al. 2007; Kochzius et al. 2003).

**Rapid Communication**

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**Abstract**

Devil firefish *Pterois miles* (Bennett, 1828) is a species native to the Indo-Pacific that along with *Pterois volitans* (Linnaeus, 1758) has been invading the western Atlantic since the 1980’s. Morphological characters, life cycle, habits, and dispersal potential of this species are very similar to those of *Pterois volitans*, to such extent that its taxonomic classification remains controversial. For example, the USGS database lists two species (*Pterois volitans/miles*) as a single one. Therefore, the probability of both species having been captured and confused is high because their identification by meristics and morphometrics is problematic. As a part of our investigation in genetic connectivity of invasive lionfish, we collected 77 specimens from Chinchorro Bank, Mexico. Identifying the samples by analysis of partial mtDNA cyt b sequences, we found that one sample corresponded to *Pterois miles*. The sequence of this specimen had 100% similarity to the sequence of *Pterois miles* specimens collected off the coast of North Carolina in 2004. This indicates that the species has extended its distribution into the Caribbean basin, but its current geographical distribution is unknown. Our results show that the presence of *Pterois miles* in the Caribbean appears low, approximately 1.3% of that of *Pterois volitans*. This study reveals the progress of the invasion of *Pterois miles* in the Caribbean and advocates for genetically confirmed identification and management of *Pterois* species.

**Key words:** *Pterois volitans*, invasive species, cyt b, Coral reefs

**Introduction**

Devil firefish *Pterois miles* (Bennett, 1828) is a species native to the Indo-Pacific, described by Bennett in 1828 (Schultz 1986). Historically, there have been several discussions over the taxonomic status of *Pterois volitans* (Linnaeus, 1758) and *Pterois miles* (Smith 1957; Beaufort and Briggs 1962; Randall 1983). However, the most recent taxonomic treatment defines them as separate species based on statistical analysis of meristic and morphometric characters (Schultz 1986). A genetic analysis revealed that these two species diverged from a common ancestor 2.4 to 8.3 million years ago, and the separation of their lineages is relatively recent (Kochzius et al. 2003). Morris et al. (2011) observed that both species have similar morphological characteristics, life cycles, habits, and dispersal potential. These similarities may cause confusion in the identification of the two species and hinder population studies. Molecular studies based on the use of mitochondrial DNA are very useful in addressing species’ identification (Freshwater et al. 2009a; Hamner et al. 2007; Kochzius et al. 2003).
The invasion of _Pterois_ species has been remarkably rapid in the Western Atlantic. Since first reported in 1985 (Schofield 2009), _Pterois volitans_ has successfully established itself on most Caribbean reefs, with a geographical distribution that ranges from the coast of New York to Brazil (Ferreira et al. 2015). Its ability to tolerate low salinity, shallow to deep depths, and long periods of fasting make the probability of invasion success high in a variety of marine and coastal environments (Kimball et al. 2004). Similarly, _Pterois miles_ also has a history as an invasive species. During the 1950s, _Pterois miles_ invaded the Mediterranean Sea via the Suez Canal (Golani and Sonin 1992). Its presence in the Western Atlantic was first confirmed in 2004 (Hamner et al. 2007).

To date, genetic evidence had shown that _Pterois miles_ was restricted to the east coast of the United States and Bermuda (Betancur-R et al. 2011; Freshwater et al. 2009b). However, _Pterois miles_ is morphologically similar to _Pterois volitans_, and most individuals collected in the Caribbean have not been analyzed using molecular markers to confirm species identification. The main objective of this study was to determine if the Devil firefish has expanded its distribution into the Caribbean. Our findings provide new insights into the population distribution of the two invasive _Pterois_ species.

**Methods**

We collected 77 specimens of _Pterois_ spp. from the Chinchorro Bank Reef (Figure 1) by scuba diving and hand spears as part of our regional genetic connectivity study at LEEAC (Laboratorio de Ecología de Ecosistemas de Arrecifes Coralinos, CINVESTAV-Merida Unit). Measurements of fish length and weight were obtained using a standard protocol (Hubbs and Lagler 1958). Additionally, a piece of muscle from the caudal peduncle of each specimen was collected, preserved individually in 70% alcohol, and stored at 4 °C for subsequent analysis in the laboratory.

Genomic DNA was extracted from muscle tissue using the DNeasy Blood and Tissue Kit (Qiagen-69506) following the manufacturer’s instructions. A fragment of the mtDNA-cyt _b_ was amplified by PCR reaction using cytb L (Schmidt and Gold 1993) and R1063 (Hamner et al. 2007) primers, under the conditions described in Hamner et al. (2007). The cyt _b_
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**Figure 3.** Phylogenetic tree generated using the Neighbor-Joining method (Saitou and Nei 1987), with sum branch lengths = 0.136082 (branch lengths noted above each branch).

A phylogenetic tree was generated using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in units of the number of base substitutions per site. The analysis involved 43 nucleotide sequences. Codon positions included were 1st+2nd+3rd+noncoding (all gaps and missing data were eliminated), resulting in 726 bp in the final dataset. All evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

A fragment obtained was purified with QIAquick PCR Purification Kit (Qiagen-28106) and sent for sequencing at the Clemson University Genomics Institute, (CUGI, USA). The generated sequences were compared and species identifications confirmed using the Basic Local Alignment Search Tool (BLAST) program in the GenBank public database.
Results

Of the 77 specimens collected near Chinchorro Bank, our analyses revealed 76 *Pterois volitans* and one individual of *Pterois miles*. The *P. miles* specimen was found in a place known as “Baliza” (18°35′21.00″N; 87°24′58.70″W; Figure 1) at a depth of 27 m, and was an adult (Figure 2). In comparing specimens of similar size, morphometric measurements showed strong similarity between the two *Pterois* species (Table 1). The partial mtDNA-cyt b sequence generated from the *Pterois miles* specimen was 783 bp (Genbank accession no. KU833279). This sequence was 99% similar to the complete mitochondrial genome of a Red Sea *Pterois miles* specimen (Genbank accession no. LK022697.1), 100% similar to the partial cyt b sequence from North Carolina specimens (Genbank accession no. EF209676.1), but only 94% similar to the partial cyt b sequence haplotype AA of *Pterois volitans* (Genbank accession no. DQ482606.1). The phylogenetic tree showed that the sequence KU833279 corresponded to the *Pterois miles* clade (Figure 3). Average genetic distance (Kimura 2-parameter model) between *Pterois miles* and *Pterois volitans* was 0.075.

Discussion

The invasion of lionfish into the Western Atlantic and the Caribbean has been unprecedented and rapid (Schofield 2009). While *Pterois volitans* has been reported widely, *P. miles* has gone nearly undetected, except for a few records in North Carolina and Bermuda (Hamner et al. 2007; Freshwater et al. 2009a; Betancur-R et al. 2011). To date, *P. miles* had never been documented in the Caribbean. And prior to this study, there had been only one lionfish reported anecdotally from Chinchorro Bank, but the record was without supporting evidence, and the individual was not identified to species (USGS 2016). Our results provide the first scientifically verified and molecularly identified records of *P. volitans* and *P. miles* from Chinchorro Bank, and the first confirmed report of *P. miles* in the Caribbean.

The presence of *P. miles* in the Mexican Caribbean is a significant expansion of its previously known distribution along the east coast of the United States and Bermuda (Betancur-R et al. 2011). There are two reasons why this species may have gone undetected until now. First, it is extremely difficult to differentiate *P. miles* and *P. volitans*, given their striking morphological similarity. The USGS-NAS (2016) lists the two species together as “*Pterois volitans/miles*” for this very reason. The only way to identify the species in their non-native range with accuracy and consistency is through molecular analysis (Freshwater et al. 2009a, 2009b; Hamner et al. 2007) as demonstrated here by our findings. Second, based on the sample size of lionfish collected from Chinchorro Bank (77 individuals), the abundance of *P. miles* may be very low (1.3%) relative to that of *P. volitans*. It remains unclear whether this result reflects the persistence of *P. miles* at small population levels or alternatively, the recent arrival of a species that has yet to increase in abundance.

Additional molecular studies are needed to fully understand each species distribution, the connectivity between populations, and the dynamics of dispersal throughout the invaded range. For example, our cyt b results show that the mitochondrial haplotype of the sample found in Chinchorro Bank corresponds 100% to the cyt b sequences of *P. miles* collected in North Carolina. Although it remains uncertain, the arrival of this species to Chinchorro Bank may be highly influenced by currents and mesoscale processes that have fostered larvae transport, slowly broadening the distribution of both species.

Acknowledgements

We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for financial support (Grant 215434) and the National Commission of Natural Protected Areas (CONANP) for logistical support in the sampling at Chinchorro Bank, as well as ECORED (194539). Our special gratitude to the staff of Banco Chinchorro Biosphere Reserve and to the fishermen who participated in the collection of samples. A special mention to Javier Salas and his XTC Dive Center team who provided us with the boat and diving equipment. We are much obliged to Dr. D. Wilson Freshwater for his valuable contributions, suggestions, and critical review of the manuscript. The authors would also like to thank anonymous reviewers for improving the manuscript.

<p>| Table 1. Morphometric measurements of <em>Pterois miles</em> and <em>P. volitans</em> of similar size. NA = data not available due to loss during capture. |</p>
<table>
<thead>
<tr>
<th>Morphological traits</th>
<th><em>P. miles</em></th>
<th><em>P. volitans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.576 kg</td>
<td>0.562 kg</td>
</tr>
<tr>
<td>Total length</td>
<td>36.5 cm</td>
<td>36.6 cm</td>
</tr>
<tr>
<td>Fork length</td>
<td>78 %</td>
<td>76.5 %</td>
</tr>
<tr>
<td>Head length</td>
<td>25.6 %</td>
<td>25.7 %</td>
</tr>
<tr>
<td>Pectoral fin length</td>
<td>41.9 %</td>
<td>42.4 %</td>
</tr>
<tr>
<td>Length of the pelvic fins</td>
<td>29 %</td>
<td>30 %</td>
</tr>
<tr>
<td>Max body height</td>
<td>29.3 %</td>
<td>22.7 %</td>
</tr>
<tr>
<td>Min body height</td>
<td>7.2 %</td>
<td>7.7 %</td>
</tr>
<tr>
<td>Max height of anal fin</td>
<td>19.3 %</td>
<td>14.1 %</td>
</tr>
<tr>
<td>Length of dorsal fin</td>
<td>NA</td>
<td>13.9 %</td>
</tr>
<tr>
<td>Length second dorsal fin</td>
<td>23.4 %</td>
<td>16.8 %</td>
</tr>
<tr>
<td>Eye diameter</td>
<td>4.1 %</td>
<td>4.3 %</td>
</tr>
<tr>
<td>Length of mouth</td>
<td>9.8 %</td>
<td>10.2 %</td>
</tr>
<tr>
<td>Snout length</td>
<td>14.1 %</td>
<td>13.5 %</td>
</tr>
</tbody>
</table>

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