Phylogenetic analysis shows the general diversification pattern of deep-sea notacanthiforms (Teleostei: Elopomorpha)

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Abstract

The Notacanthiformes is an ancient group of deep-sea ray-finned fishes comprising 27 species in two families; Halosauridae and Notacanthidae. Although many studies have tried to reconstruct the phylogenetic relationships among the major clades of Elopomorpha, little is known about the evolutionary history of notacanthiforms. Molecular and morphological data were used to test previous hypotheses regarding the phylogenetic relationships among notacanthiform taxa, and to unravel the origin and evolution of this group. The molecular analyses of notacanthids showed similar results to those previously obtained employing osteological data, which proposed the existence of the Lipogenyinae (*Lipogenys*) and Notacanthinae (*Notacanthus* + *Polyacanthonotus*) subfamilies. Nevertheless, when the external morphology data is considered *Lipogenys* is more related to *Notacanthus* than *Polyacanthonotus*. The analyses could not fully resolve the inner relationships of the halosaurids. The time-calibrated tree of the order Notacanthiformes shows a long process of

diversification spanning from the upper Cretaceous, to 50 million years after the K-Pg extinction, with the gradual emergence of all the modern families and genera of the group. This is the first specific phylogeny of the order Notacanthiformes, combining different analyses and data in order to obtain a wider perspective of the evolution and diversification of this group of fishes.

Keywords: Evolution; Notacanthiformes; Speciation; DNA, Bayesian

Introduction

Notacanthiformes Goodrich, 1909, is an order of deep-sea fishes most of them distributed worldwide from the continental slope to the abyssal plain (Nelson 2016). They are characterised by an eel-like body, premaxilla and maxilla bordering the upper jaw, with a connective tissue structure intercalated between pterygoid and maxilla, and a posteriorly directed spine on the dorsal edge of the rear of maxilla (Nelson 2016). Despite order Notacanthiformes comprises only 27 species classified into two families, they show a wide range of feeding strategies, like hyperbenthic and detritus feeders, epifaunal browsers, and megafaunal croppers (Drazen & Sutton, 2017, Nelson 2016).

Due to the common presence of the leptocephalus larvae, the order Notacanthiformes has been included in the superorder Elopomorpha. The latter comprises about 986 species organised in four orders, 24 families and 169 genera, and is one of the four crown teleost groups, together with Osteoglossomorpha, Otocephala and Euteleostei (Nelson et al. 2016). The phylogenetic relationships of Elopomorpha have been established with time-calibrated phylogenies, estimating the origin at about 200 million years ago, considering it the sister group of the teleosts (Broughton et al. 2013, Near et al. 2012).

The relationships among the four recognised orders of Elopomorpha: Albuliformes (bonefishes), Notacanthiformes (halosaurs and spiny eels), Anguilliformes (eels) and Elopiformes (tarpoons and ladyfishes) have been controversial historically (Chen et al. 2014). Their morphology-based systematics, from the late 1960s and early 1970s, was reviewed to obtain a phylogenetic analysis of 56 morphological characters (Forey et al. 1996). The results showed that Elopiformes is the sister lineage that forms the earliest diverging clade of elopomorphs. Later analyses based on molecular data were in accordance (Chen et al. 2014, Near et al. 2012). However, in phylogenetic studies of the superorder Elopomorpha the

notacanthiforms are represented by only a few species, without even including all recognised genera (Chen et al. 2014, Dornburg et al. 2015).

Despite the great efforts made to solve the relationships among these orders, little has been done to establish the inner relationships among notacanthiforms. A phylogeny based on osteological characters was carried out at genus level showing *Lipogenys* as the sister lineage of all the rest of the Notacanthidae family, proposing the creation of the subfamily *Lipogenyinae* (Kanehira et al. 2012). Lately, new studies based on notacanthiform species have been published: a DNA barcoding analysis highlighted the possibility of unknown taxa (Barros-García et al. 2016), in agreement with other molecular analyses that showed the possibility of cryptic species (Poulsen et al. 2017). On the other hand, an integrative taxonomy approach to North Atlantic halosaur species showed the absence of incongruities between molecular and morphological data in their systematics (Bañón et al. 2016). Therefore, in this investigation, a first assessment of the phylogenetic relationships among notacanthiforms is presented using a combination of molecular (one nuclear and three mitochondrial markers) and morphological data (combining osteological, biometric, and meristic data) in an attempt to shed light on this small but ancient branch of the tree of life. Furthermore, a time-calibrated tree clarifies the timing of notacanthiforms diversification, with ecological features offering a possible explanation.

2. Materials and methods

2.1 Sample collection

All notacanthiform individuals were morphologically identified to species level following taxonomic literature (as used in Barros-García et al. 2016). Twenty individuals representing all families and genera of the order Notacanthiformes were used (Table 1).

2.2 Molecular data

Genomic DNA was extracted following Barros-García et al. (2016). The three mitochondrial and one nuclear genes used for the analyses and their primers are listed in Table 2. PCR conditions were as previously described (Barros-García et al. 2016) with annealing temperatures ranging from 55 °C (*COI*, *16S*, *12S*) to 59 °C (*ZIC1*). Sequencing was performed by Macrogen Inc., Korea using the BigDye sequencing protocol and an Applied Biosystems 3730xl genetic analyser with the same primers used in the PCR. The electrophoretograms were visually revised and edited with the software Chromas ChromasPro 2.6 (Technelysium, Tewantin, Australia). The protein-coding sequences were translated to amino acid and no stop codons were found.

MAFFT (Kazutaka & Standley 2016) was used to produce the individual mtDNA marker alignments, including the Anguilliformes (Notacanthiformes sister group) and the outgroup species. The former were selected and retrieved from GenBank and consisted of six species representing the order (Table 1). The outgroup consisted of *Megalops cyprinoides* (Broussonet 1782) and *Megalops atlanticus* (Valenciennes, 1847) (Elopiformes), *Albula glossodonta* (Forsskål, 1775) and *Pterothrissus gissu* (Hilgendorf 1877) (Albulidae), being Elopiformes an earlier branch of the elopomorphs, and Albuliformes a more closely-related taxa to Notacanthiformes (Kanehira et al. 2012) (Table 1). The final concatenate mtDNA data matrix was 1596 bp long and all individual sequences were deposited in GenBank (Table 1).

Polymorphic positions found in *ZIC1* corresponding to heterozygous specimens were coded according to IUPAC. The phased haplotypes were inferred probabilistically through the Bayesian algorithm implemented in PHASE v2.1.1 (Stephens et al. 2001, Stephens & Donnelly, 2003), using SeqPHASE (Flot 2010) to prepare the input files. Software PHASE was run, under default values, three times with different random seeds and checked for haplotype estimation consistency. A threshold of 0.75 posterior probability was used to accept a given haplotype phase reconstruction. All the haplotypes obtained (final length 834 bp) were submitted to GenBank (Table 1).

2.3 Morphological data

Three different types of data were combined in morphological analysis; osteological, biometric and meristic. The osteological data regarding notacanthiforms and both outgroups were retrieved from previous investigations (Kanehira et al. 2012; Shelyagin 2010). The material examined for biometric and meristic data is listed in Table S1. The data from *Megalops* and *Pterothrissus* were taken from Dolganov et al. 2008 and Hidaka et al. 2017. A total of 16 biometric measurements were used after being transformed relative to standard length (% SL). Four out of the latter: preorbital, postorbital, interorbital length and eye diameter were also transformed relative to head length (% HL), obtaining the final 20 measurements (Appendix A). Different coding strategies for the continuous characters were tested in order to obtain a correct representation of the data. The chosen strategies and the full description of all the characters used are summarized in the supplemental material (Appendix A). The final codified matrix consisted of 56 columns comprising 20 biometric, 6 meristic, and 30 osteological characters (Table S2).

2.4 Phylogenetic analyses

For the molecular data, the best partitioning scheme was estimated with PartitionFinder2 (Lanfear et al. 2016) spanning the range from a single partition for the entire alignment to each gene and codon position treated as a partition. The saturation level of the partitions obtained was assessed with DAMBE 6 (Xia 2017) obtaining high levels of saturation for the third *COI* position. The most appropriate nucleotide substitution models were selected using jModelTest 2.1.8 (Darriba et al. 2012) based on the Akaike Information criterion (AIC; Akaike, 1973) for every partition. The phylogenetic trees were constructed using Bayesian Inference (BI), performed in MrBayes 3.2 (Ronquist et al. 2012). The optimal methods and the best partitioning scheme chosen are summarized in Table 2. Four Markov chains were employed in two different runs of 10×10^6 generations sampling every 1000 generations for a dataset of 10000 trees. No stop value was fixed and the burn-in of the first 25% of the trees was used. The software Tracer 1.6 (Rambaut et al. 2014) was employed to verify the convergence of the chains and the values of the estimations of the parameters.

The molecular dataset, (*COI*+*I6S*+*I2S*+*ZIC1*) including 22 sequences (2404 bp), was combined with the 56 morphological characters, totalizing 2460 positions of concatenated dataset. The BI analyses were carried out as described above using the commands: DIMENSIONS NCHART=2460; FORMAT datatype=mixed (DNA; 1-2404, Standard: 2405-2460).

Divergence times of notacanthiform lineages were estimated using an uncorrelated lognormal clock model in BEAST 2.4.6 (Bouckaert et al. 2014). A birth-death process was used in order to branch the tree with initial birth rate = 1.0 and death rate = 0.5. The molecular data was used with the nucleotide substitution models and partitions as mentioned before. Two calibration points were selected as priors for divergence time estimates, both based on Dornburg et al. 2015: most recent common ancestor (MRCA) of Elopomorpha, † *Anaethalion zapporum* resolved as a member of crown group Elopomorpha, from the Rögling formation (top of the Kimmeridgian age, dated to 152.1 ± 09 Ma); MRCA Anguilliformes and Notacanthiformes, † *Angullavis bathshebae*, † *Hayenchelys germanus* resolved as stem anguilliforms, from the Hajoula Lagerstätte (Lebanese Cenomanian deposits, dated approximately 98.0 Ma). Three MCMC replicates of 20 million generations each, sampling every 1000 generations, were used. Effective Sample Size (ESS) values were calculated for each parameter to check the adequate mixing of the MCMC ($ESS > 200$) and revised with Tracer v1.6 (Rambaut et al. 2014). Tree files from the three replicates were combined in LogCombiner v2.4.6 (Bouckaert et al. 2014) after a burn-in of 25%. The Maximum Clade Credibility Tree was estimated with TreeAnnotator v. 2.4.6 (Bouckaert et al. 2014). Four different molecular datasets were used in order to test the effect of partition strategy and saturation levels in the posterior age estimates of main nodes; Partition Finder suggested partition with and without saturated positions, and all the markers concatenated with and without saturated positions.

171

172 3. Results

173 The Bayesian trees obtained from the molecular (a) and the concatenated data (b) are shown in
174 Figure 1. The obtained topologies showed the monophyly of the order Notacanthiformes, with
175 high support values in all nodes (Fig. 1a, b). In both trees, two major clusters representing the
176 Halosauridae and Notacanthidae families are recovered. The Halosauridae branch is further
177 divided in three, comprising the genera: *Aldrovandia*, *Halosaurus* and *Halosauropsis*, including
178 6 species and 12 individuals. Although both trees recovered the monophyly of Halosauridae
179 (0.99 and 0.83 support values respectively), the relationships among these genera are poorly
180 supported (< 0.75) and remain unclear in both phylogenies. In the case of the *Aldrovandia*
181 cluster, *A. affinis* and *A. oleosa* showed a closer relationship compared to *A. phalacra*. On the
182 other hand, the Notacanthidae cluster showed different well supported topologies depending on
183 the data type (molecular vs concatenated), suggesting two different phylogenetic hypotheses for
184 this family. In the molecular tree (Fig. 1a) two major clusters were retrieved, the first including
185 *L. gillii* and the second the *Notacanthus* and *Polyacanthonotus* genera, both well supported.
186 However, the phylogeny obtained employing the concatenated data (Fig. 1b) grouped *L. gillii*
187 with the *Notacanthus* species, while *P. rissoanus* remained alone.

188 The estimates of split times were calculated for highly supported nodes in the final tree, inferred
189 from the optimal strategy with saturated partitions removed using BEAST v.2.4.6 (Fig. 2). The
190 tree showed the monophyly of Elopiformes and that the time estimation for the split between the
191 two species of *Megalops* was 26.86 mya (95% HPD: 8.8-48.94). There is an uncertainty
192 regarding the order Albuliformes and the relationships between *Albula* and *Pterothrissus*. The
193 origin of the crown group of Anguilliformes was dated in 94 mya (95% HPD: 72.93-114.7)
194 showing two highly supported nodes; the *Anguilla* + *Serrivomer* clade estimated in 66 mya
195 (95% HPD: 39.67-90.47) and the node of the rest of Anguilliformes (95% HPD: 54.25-100).

196 The analysis showed the monophyly of the order Notacanthiformes, resolving the inner
197 relationships of this taxa (BPP > 0.9) with the exception of *Halosauropsis*, which showed
198 moderate support (BPP=0.83). The first split in the notacanthiform lineage happened between
199 the families Halosauridae and Notacanthidae, 73.49 mya (95% HPD: 49.48-97.09) between the
200 second half of the Cretaceous and the early Eocene. The diversification of the Notacanthidae
201 major lineages seems to have occurred gradually from the last millions of years of the
202 Cretaceous to the early Miocene, with the *Lipogenys* clade separated from the rest at around 50
203 mya (95% HPD: 29.62-72.41), and the genera *Notacanthus* and *Polyacanthonotus* splitting
204 31.99 mya (95% HPD: 16.66-49.56). Finally, the separation between *N. bonaparte* and *N.*
205 *chemnitzii* seemed to have happened at 11.31 mya (95% HPD: 3.97-20.38). The diversification

of the Halosauridae family, with the appearance of its major lineages, took place at the same time as Notacanthidae, appearing around 59.13 mya (95% HPD: 38-81.81). The phylogenetic relationships among Halosauridae genera showed a moderate support to a closer relationship between *Halosauropsis* and *Halosaurus*, whereas the nodes showing the common ancestor in *Aldrovandia* dated 15.89 mya (95% HPD: 6.46-27.66) and 6.98 mya (95% HPD: 2.29-12.69) between *A. affinis* and *A. oleosa*. The split in the represented *Halosaurus* species, *H. ovenii* and *H. johnsonianus*, was estimated in 24.22 mya (95% HPD: 10.69-39.49).

The time estimation comparisons (Fig. 2) showed more ample confidence intervals and slightly more recent time estimates for those analyses without saturated partitions. For the crown node of Notacanthiformes the values varied from 73.2 mya (95% HPD: 47.8-99.5) and 73.5 (95% HPD: 49.5-97) from the datasets without saturated partitions to 76.2 (95% HPD: 57.3-96.1) and 79.5 (95% HPD: 56.6-103.6) when the saturated partitions were taken into account. Similar results were obtained for the Halosauridae and Notacanthidae families. In the former, the analysis without saturated partitions showed an estimation of 59 mya (95% HPD: 38-81.8) in partitioned data, and 61.7 (95% HPD: 36.6-85.1) in the concatenated data, whereas saturated partitions retrieved values of 64.2 (95% HPD: 43.8-86) and 62.3 (95% HPD: 44.6-81.4) respectively. The crown node for the Notacanthidae family showed similar estimations for non-saturated data, 49 mya (95% HPD: 27.3-72.7) and 50.2 (95% HPD: 29.6-72.4), and when saturated partitions were used, 54.2 mya (95% HPD: 37.4-72.2) and 57 (95% HPD: 37.7-77.7), respectively.

4. Discussion

The phylogenies obtained in this investigation include representatives of all known genera of notacanthiforms and show their monophyletic nature at several taxonomical levels (order, family and genus). These results agree with those obtained in previous phylogenetic studies where notacanthiforms were partially represented among a huge number of fish taxa (Betancur et al. 2017; Chen et al. 2014; Dornburg et al. 2015).

However, the open question about the evolutionary relationships among Halosauridae genera *Aldrovandia*, *Halosauropsis* and *Halosaurus* remain unsolved due to the partial loss of node support among them, a fact also previously observed (Chen et al. 2014). The diversity of data, including mitochondrial and nuclear markers and morphological traits, may suggests the possibility of three simultaneous speciation events from the same common ancestor, a so-called “hard” polytomy.

The relationships among the three major lineages in the Notacanthidae family showed two different hypotheses depending on the type of data used. First, the molecular data suggests

Lipogenys as the sister lineage of *Notacanthus* and *Polyacanthonotus*, as previously reported in an osteological-based phylogeny. There, the *Lipogenys* clade is supported by two osteological apomorphies and the *Notacanthus* and *Polyacanthonotus* lineage by three synapomorphies (Kanehira et al. 2012). Distinctly, the concatenated data show *Polyacanthonotus* as the sister lineage of *Lipogenys* and *Notacanthus*. Because the molecular analyses arrive to the same conclusion as the osteological ones, the only explanation for this discrepancy is the addition of new biomeristic traits to the already existing osteological data. This would suggest that the external morphology of *Lipogenys* is more similar to *Notacanthus* as they share similar ecological strategies feeding on benthic organic matter or sessile organisms, while *Polyacanthonotus* are active predators of crustaceans more than a shared common ancestor (Romeu et al. 2016; Templeman, 1973). Therefore, the combination of the molecular and osteological data supports the subfamily Lipogenyinae as the sister lineage of Notacanthinae, as previously proposed (Kanehira et al. 2012).

The time calibrated analyses has dated the diversification of the notacanthiforms between the upper Cretaceous and early Eocene (95% HPD: 49.48-97.09), a younger estimation than others previously obtained (Dornburg et al. 2015, Santini et al. 2013). This difference could be related to the increase in the number of specific taxa employed, which may have improved the phylogenetic inference (Dornburg et al. 2015). In this period three main events happened: the K/Pg extinction (65 mya) and two different diversifications for actinopterygians; the Cenomanian (about 100 mya) and the Paleocene-Eocene interval (66-33.9 mya), both the two most exceptional radiations among vertebrates (Guinot & Cavin, 2016). However, the broad confidence interval does not allow us to establish a direct relationship among any of them and the origin of notacanthiforms. Nevertheless, the fossil record of halosaurs shows that their typical morphology was already present in the upper Cretaceous (89.3-85.8 mya), suggesting that the split between Halosauridae and Notacanthidae happened before the K/Pg extinction (Dornburg et al. 2015). The confidence intervals dating the diversification of modern notacanthiform families span from the upper Cretaceous to the early Oligocene, partially overlapping with each other and with the crown node of the notacanthiforms. Unlikely, the time estimation of Halosauridae showed older values than others previously obtained (Dornburg et al. 2015). Our study has obtained for the first time a complete genus level timetree for the Notacanthidae family. The results also support the subfamily Lipogenyinae as the sister lineage of Notacanthinae, as previously proposed (Kanehira et al. 2012).

Despite the wide time intervals obtained, our results show a higher effect of saturation than the partition strategy in the time estimation, with older estimations when saturated partitions are included in the analysis. When saturation is not taken into account, the use of mitochondrial markers led to produce older ages than the nuclear ones (Dornburg et al. 2014).

The order Notacanthiformes shows a long process of diversification spanning from the upper Cretaceous, about 100 million years ago, to 50 million years after the K-Pg extinction, with the gradual emergence of all the modern families and genera of the group. This study represents the first specific phylogeny of notacanthiforms, combining different analyses and data to obtain a wide perspective of the evolution and diversification of this old group of fishes. We provide strongly supported results showing *Lipogenys* as the sister lineage of the rest of the notacanthidae genera, although we cannot fully resolve the inner relationships of the Halosauridae family. Future analyses, probably including extinct lineages, will be required in order to achieve a complete phylogenetic hypothesis of the order Notacanthiformes and resolve this branch of the Tree of Life.

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Figures

Figure 1:

Phylogenetic trees constructed using the BI method. a) tree based on the molecular dataset (COI+16S+12S+ZIC1). b) tree based on the concatenated dataset (molecular dataset + morphological matrix). Support values for each node are included (values < 0.75 are not shown).

Figure 2:

Time-calibrated phylogeny of notacanthiforms based on one nuclear and three mitochondrial markers and two temporal constraints. Bars represent the posterior distribution of divergence-time estimates. Black dots represent node supports ≥ 0.95 . Red dots show calibration points in the tree. The time-calibrated tree is scaled in millions of years. The red line shows the Cretaceous/Paleogene extinction event and the green areas the known actinopterygian diversification events. Illustrations were obtained from Raymond Simpson (<http://watlfish.com/>) and the NOAA Photo Library for Halosauridae and Notacanthidae families respectively. Inset: Comparison of ages estimated for order Notacanthiformes, Halosauridae and Notacanthidae families; Red, PartitionFinder partition with saturated positions. Blue, PartitionFinder partition without saturated positions. Violet, all concatenated with saturated positions. White, all concatenated without saturated positions

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Table 1

List of specimens examined in this study and GenBank accession numbers for ZIC1 and mitochondrial genes (12S, 16S, COI) collected.

Order	Family	Species	ID	12S	16S	COI	ZIC1
Albuliformes	Albulidae	<i>Albula glossodonta</i>	NA	NC_005800.1	NC_005800.1	NC_005800.1	JX191248.1
Albuliformes	Albulidae	<i>Pterothrissus gissu</i>	NA	NC_005796.1	NC_005796.1	NC_005796.1	KJ910156.1
Anguilliformes	Anguillidae	<i>Anguilla rostrata</i>	NA	KJ564206.1	KJ564206.1	KJ564206.1	EU001889.1
Anguilliformes	Congridae	<i>Conger japonicus</i>	NA	NC_027186.1	NC_027186.1	NC_027186.1	JX255601.1
Anguilliformes	Congridae	<i>Heteroconger hassi</i>	NA	NC_013629.1	NC_013629.1	NC_013629.1	JX191254.1
Anguilliformes	Derichthyidae	<i>Derichthys serpentinus</i>	NA	NC_013611.1	NC_013611.1	NC_013611.1	FJ906633.1
Anguilliformes	Ophichthidae	<i>Myrichthys maculosus</i>	NA	NC_013635.1	NC_013635.1	NC_013635.1	NA
Anguilliformes	Serrivomeridae	<i>Serrivomer beanii</i>	NA	NC_013627.1	NC_013627.1	NC_013627.1	FJ906634.1
Elopiformes	Megalopidae	<i>Megalops atlanticus</i>	NA	NC_005804.1	NC_005804.1	NC_005804.1	JX191251.1
Elopiformes	Megalopidae	<i>Megalops cyprinoides</i>	NA	NC_005799	NC_005799	NC_005799	KJ910161
Notacanthiformes	Halosauridae	<i>Aldrovandia affinis</i>	NHA001	MF156145	MF156165	KP845174	MF156197
Notacanthiformes	Halosauridae	<i>Aldrovandia affinis</i>	NHA002	MF156146	MF156166	KP845175	MF156198
Notacanthiformes	Halosauridae	<i>Aldrovandia oleosa</i>	NHO001	MF156147	MF156167	KP845185	MF156191/MF156192
Notacanthiformes	Halosauridae	<i>Aldrovandia oleosa</i>	NHO002	MF156148	MF156168	KP845184	MF156193
Notacanthiformes	Halosauridae	<i>Aldrovandia phalacra</i>	JBD001	MF156157	MF156177	KP845193	MF156194/MF156195
Notacanthiformes	Halosauridae	<i>Aldrovandia phalacra</i>	JBD002	MF156158	MF156178	KP845194	MF156196
Notacanthiformes	Halosauridae	<i>Halosauropsis macrochir</i>	NHH001	MF156141	MF156161	KP845201	MF156184

Notacanthiformes	Halosauridae	<i>Halosauropsis macrochir</i>	NHH002	MF156142	MF156162	KP845196	MF156185
Notacanthiformes	Halosauridae	<i>Halosaurus johnsonianus</i>	NHI001	MF156149	MF156169	KP845203	MF156190
Notacanthiformes	Halosauridae	<i>Halosaurus johnsonianus</i>	NHI002	MF156150	MF156170	KP845204	NA
Notacanthiformes	Halosauridae	<i>Halosaurus ovenii</i>	NHU001	MF156143	MF156163	KP845208	MF156186/MF156187
Notacanthiformes	Halosauridae	<i>Halosaurus ovenii</i>	NHU002	MF156144	MF156164	KP845209	MF156188/MF156189
Notacanthiformes	Notacanthidae	<i>Lipogenys gillii</i>	TSN001	MF156151	MF156171	KP845211	MF156199
Notacanthiformes	Notacanthidae	<i>Lipogenys gillii</i>	TSN002	MF156152	MF156172	MF156183	MF156200
Notacanthiformes	Notacanthidae	<i>Notacanthus bonaparte</i>	NBO001	MF156153	MF156173	KP845212	MF156201/MF156202
Notacanthiformes	Notacanthidae	<i>Notacanthus bonaparte</i>	NBO002	MF156154	MF156174	KP845234	MF156203
Notacanthiformes	Notacanthidae	<i>Notacanthus chemnitzii</i>	NNN001	MF156159	MF156179	MF156181	MF156204
Notacanthiformes	Notacanthidae	<i>Notacanthus chemnitzii</i>	NNN002	MF156160	MF156180	MF156182	MF156205
Notacanthiformes	Notacanthidae	<i>Polyacanthonotus rissoanus</i>	NNP001	MF156155	MF156175	KP845235	MF156206/MF156207
Notacanthiformes	Notacanthidae	<i>Polyacanthonotus rissoanus</i>	NNP002	MF156156	MF156176	KP845236	MF156208

435

436 **Table 2**

437 Information regarding molecular data. Different markers used with their respective primers.

438 Partitions selected with PartitionFinder and substitution models obtained with jModelTest.

Gene	Primers	Expected Size	Reference	Partition	Model
<i>12S</i>	12SL/12SH	400 bp	Wang et al. 2000	12S	GTR+G+I
<i>16S</i>	16Sar-5′/16Sbr-3′	600 bp	Palumbi 1996	16S	GTR+G+I
<i>COI</i>	C_FishF1t1-C_FishR1t1	700 bp	Ivanova et al. 2007	COI_1	GTR+G+I
				COI_2	JC
				COI_3	GTR+G
<i>ZIC1</i>	ZIC1_F9, ZIC1_R967 ZIC1_F16 ZIC1_R963	850 bp	Li et al. 2007	ZIC1_1	GTR
				ZIC1_2	HKY
				ZIC1_3	HKY+I

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