

# Phylogenetic characterisation of the genus *Idiosepius* (Cephalopoda; Idiosepiidae)

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**ABSTRACT:** The family Idiosepiidae is an atypical cephalopod group; the member species are the smallest cephalopods in body size, and their phylogenetic position with regard to the other cephalopods as well as the relationships within the genus *Idiosepius* remain controversial. Currently, 8 recognized species belong to *Idiosepius*, although the taxonomic position of *I. macrocheir* and *I. thailandicus* is uncertain: their diagnostic characters closely overlap with those of *I. biserialis*. To provide further information on the phylogenetic relationships of *Idiosepius*, 4 mitochondrial loci (12S rRNA, 16S rRNA and cytochrome *c* oxidase subunits I and III) were analysed for all *Idiosepius* species and several populations. *I. macrocheir* and *I. thailandicus* nested within the African and the Indo-Pacific group of *I. biserialis*, respectively. This indicates that both species were incorrectly assigned to a single species, and that they rather represent junior synonyms of *I. biserialis*. Furthermore, the species *I. biserialis* itself exhibits considerable genetic variability: an African and Indo-Pacific region population was evident. Our results revealed 2 new aspects of *Idiosepius*: a population of *I. biserialis* from Japan appeared to be closely related to *I. paradoxus*, a species with 4 rows of suckers on the tentacular club. In contrast, *I. paradoxus* from Okinawa Island, also considered a 4-rowed species, showed strong congruence on the phylogenetic and taxonomic level with the 2-rowed species *I. biserialis*. This congruence leads to the hypothesis that climatic conditions, rather than habitat preference or geographical barriers, support the structuring of *Idiosepius* populations.

**KEY WORDS:** Holotype analysis · Classification · Species characterisation · Mollusca

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## INTRODUCTION

The genus *Idiosepius* Steenstrup, 1881 comprises the smallest species among cephalopods, with a mantle length less than 6 mm in females and 3 mm in males (von Byern & Klepal 2010). A conspicuous morphological character of this family is the adhesive organ (also known as the adhesive gland), which is restricted to the posterior part of the dorsal mantle and fin region (Steenstrup 1881, Sasaki 1921, von Byern et al. 2008, Cyran et al. 2011).

Idiosepiids have been studied for many interesting features, including life history strategies, physiology, behaviour and adhesive organ morphology (Nabhitabhata 1998, Kasugai 2000, Shigeno & Yamamoto

2002, von Byern et al. 2008, Cyran & von Byern 2010). Nonetheless, the phylogenetic placement of the Idiosepiidae remains contentious. Two competing hypotheses have been proposed. The first places the genus into the Sepiidae (Clarke 1988, von Boltzky 1995, Carlini & Graves 1999, Lindgren et al. 2004, Strugnell et al. 2005, Akasaki et al. 2006), whereas the second argues for the Teuthidae (Bonnaud et al. 1996, 1997, 2005, Carlini et al. 2000, Takumiya et al. 2005).

Moreover, the number of species that the genus *Idiosepius* comprises also remains unclear. Jereb & Roper (2005) proposed 8 species: *I. biserialis* Voss, 1962; *I. macrocheir* Voss, 1962; *I. minimus* (D'Orbigny & de Ferussac 1848); *I. notoides* Berry, 1921; *I. paradoxus*

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Ortmann, 1888; *I. picteti* (Joubin, 1894); *I. pygmaeus* Steenstrup, 1881 (= *I. pygmaeus hebereri* Grimpe, 1931) and *I. thailandicus* Chotiyaputta et al., 1991. The geographical distribution of these species is large (Fig. 1), from Japan to the Indo-Pacific region including Australia and Tasmania as well as Mozambique (incorrectly annotated as South Africa by Voss 1962).

Of these 8 species, 6 are represented in a systematic key proposed by Nesis (1987). The species are characterised by the arrangement of suckers on the tentacular club (*Idiosepius biserialis* with 2 rows, *I. notooides*, *I. macrocheir*, *I. paradoxus*, *I. picteti* and *I. pygmaeus* with 4 rows) and the number of suckers on the male hectocotylus (Nesis 1987). *I. thailandicus* was described afterwards (Chotiyaputta et al. 1991), and the holotype material of *I. minimus* is lost, thus its systematic position can no longer be verified. These 2 species are therefore missing in the key.

The discovery of *Idiosepius thailandicus* (Chotiyaputta et al. 1991) in Thai waters along with *I. biserialis* (Hylleberg & Nateewathana 1991a) raises questions about the validity of the systematic key. These 2 species resemble each other closely by a biserial sucker arrangement on the tentacular clubs and the same number of suckers on the hectocotylised arms. Chotiyaputta et al. (1991) instead proposed to differentiate the 2 species by mantle length (*I. thailandicus* is slightly smaller than *I. biserialis*) and sucker ultrastructure. It remains unresolved whether these 2

morphological features (mantle length and sucker ultrastructure) are reliable characters for other species of the genus.

To compare these features, von Byern & Klepal (2010) conducted a morphological re-examination of all taxonomic characters proposed by Nesis (1987) and Chotiyaputta et al. (1991) in all nominal *Idiosepius* species. Their results support a revision of the systematic key of Nesis (1987). Von Byern & Klepal (2010) proposed that species should be characterised by the shape of the hectocotylus and its appendages rather than by the sucker arrangement on the tentacular club. Based on this information, the systematic position of *I. thailandicus* is uncertain because it does not differ from *I. biserialis* in mantle length, form or number of suckers on the hectocotyli and tentacular clubs, or in other morphological attributes (von Byern & Klepal 2010). That evaluation also supports a revision of the systematic status of *I. macrocheir*. Based on the number of suckers on the tentacular club, *I. macrocheir* belongs to the 2-rowed rather than to the 4-rowed group; its 4-rowed arrangement appears to be an artefact of the fixation process (von Byern & Klepal 2010).

Given all of these major differences in morphological characters and systematic placement of individual species, our aim was to provide information on the phylogenetic relationship of the Idiosepiidae based on molecular data.

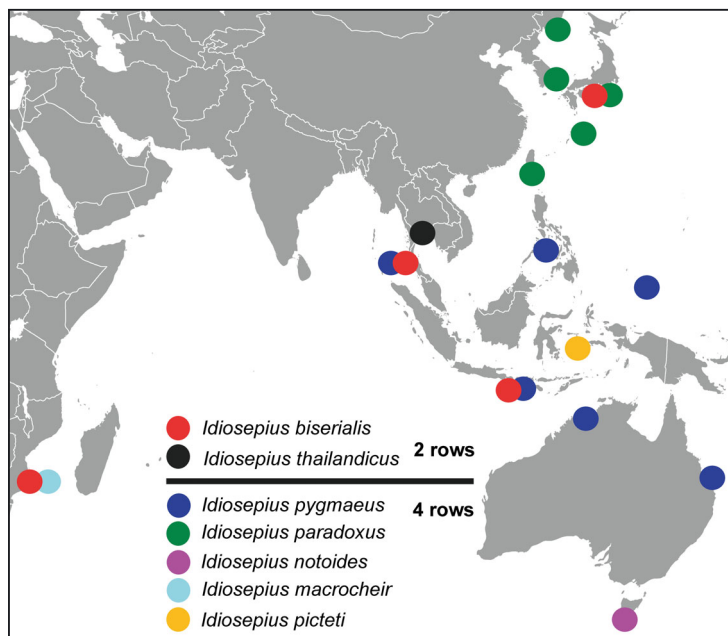


Fig. 1. Geographical distribution and collection sites for all nominal *Idiosepius* spp. according to Nesis (1987). 'Rows' refers to the arrangement of suckers on the tentacular club

## MATERIALS AND METHODS

In total, 88 specimens of 7 nominal taxa from different locations (*Idiosepius biserialis* n = 34; *I. macrocheir* n = 2; *I. notooides* n = 4; *I. paradoxus* n = 20; *I. picteti* n = 1; *I. pygmaeus* n = 21 and *I. thailandicus* n = 6) were obtained for the analysis. A detailed description of the collected specimens and their locations with latitude and longitude data as well information of all known localities are given in Table S1 in the supplement at [www.int-res.com/articles/suppl/b017p019\\_supp.pdf](http://www.int-res.com/articles/suppl/b017p019_supp.pdf)

It was possible to extract DNA from the holotype sample of *Idiosepius picteti*, and the animal was therefore used for the phylogenetic analysis. However, the long storage (>100 yr) and/or fixation (10% formalin) modified or damaged its DNA, and it was not possible to correctly allocate this species to the other representatives of the family. We retained the species in the analysis to docu-

ment our attempts and initial ideas about its possible allocations. More material or other gene loci need to be analysed to verify the systematic status of *I. picteti*.

**DNA extraction, amplification and sequencing**

Living specimens were fixed in 90% ethanol. For the DNA extraction, muscle tissue (without the epidermis and chromatophores) from the arm or mantle area was used and washed several times in distilled water to eliminate the ethanol. Total DNA was isolated with a DNeasy Tissue Kit (Qiagen). Once isolated, 1 to 10 ng of DNA were used for polymerase chain reaction (PCR), performed with MangoTaq DNA Polymerase (Bioline). Four different loci were analysed, i.e. partial sequences of the genes for 12S rRNA (386 bp in total), 16S rRNA (505 bp in total), cytochrome *c* oxidase subunit I (COI; 640 bp in total) and subunit III (COIII; 657 bp in total). Specific primer sets were used to amplify each of the 4 loci (Table 1). Additional internal primers were constructed to sequence samples of the type material. The PCR protocol is summarized in Table 2; for 16S rRNA, 2 PCR protocols were used.

PCR products were purified with the peq-Gold Cycle-pure Kit (Peqlab) and sequenced on an Applied Biosystems automated capillary sequencer (model 3130xl) using Applied Biosystems Big Dye v3.1.

**Phylogenetic analysis**

Sequences were aligned with ClustalX Version 1.83 (Thompson et al. 1997), with no gaps or ambiguities in the alignments of any sequences. All variable sites were reconfirmed by visual inspection of the sequence data

Table 1. Primers used for PCR amplification and sequencing. Primers were used in various combinations of forward (f) and reverse (r) primers to provide overlapping fragments, thus increasing the efficiency of some PCR amplifications. All possible primer combinations shown here were effective and did not lead to a primer dimerization

Primer	Sequence (5'–3')
COI	GGT CAA CAA ATC ATA AAG ATA TTG G
COI	TAA ACT TCA GGG TGA CCA AAA AAT CA
COI-140f	GTC GTT GTA ACA GCT CAC GG
COI-140r	CCG TGA GCT GTT ACA ACG AC
COI-200f	GGA TTT GGG AAT TGA TTG GTA CC
COI-200r	GGT ACC AAT CAA TTC CCA AAT CC
COI-320f	GGC AGT TGA AAG AGG GGC AG
COI-320r	CTG CCC CTC TTT CAA CTG CC
COI-480f	CGA TGA GAA GGT TTA CAA ATA GAA CG
COI-480r	CGT TCT ATT TGT AAA CCT TCT CAT CG
COI-580r	ATT GCA CCA GCA AGT ACA GG
COIII-f	CAT TTA GTT GAT CCT AGG CCT TGA CC
COIII-r	CAA ACC ACA TCT ACA AAA TGC CAA TAT
COIII-110f	CTC CAA TGA TGA CGA GAT AT
COIII-110r	ATA TCT CGT CAT CAT TGG AG
COIII-260f	CAG AAC TAG GWG CCT GTT GAC
COIII-260r	GTC AAC AGG CWC CTA GTT CTG
COIII-360f	CAG GCG TWA SGG TAA CAT GAG
COIII-360r	CTC ATG TTA CCS TWA CGC CTG
COIII-460f	CAA GCA GAR GAA TAT ATA GAA GC
COIII-460r	GCT TCT ATA TAT TCY TCT GCT TG
12S rRNA-f	AAA CTA GGA TTA GAT ACC CTA TTA T
12S rRNA-r	AAG AGC GAC GGG CGA TGT GT
12S rRNA-180f	CTG ATG ACA GAA ATA TAC AAA CTG
12S rRNA-280r	CCT TTC AGG GGA GTT TGC
16S rRNA-f	CGC CTG TTT ATC AAA AAC AT
16S rRNA-r	CTC CGG TTT GAA CTC AGA TCA
16S rRNA-100f	GAG GCT AGA ATG AAT GGT TTG ACG
16S rRNA-100r	CGT CAA ACC ATT CAT TCT AGC CTC
16S rRNA-200f	GAC GAG AAG ACC CTA GTG AGC
16S rRNA-200r	GCT CAC TAG GGT CTT CTC GTC
16S rRNA-360f	GTT ACC ATA GGG ATA ACA GCG
16S rRNA-360r	CGC TGT TAT CCC TAT GGT AAC

and verified on both strands. GenBank accession numbers for all sequences are provided in Table S2 in the supplement. A total of 90 aligned sequences of *Idiosepius* were used, with *Sepia officinalis* (AB 193808.1; AB 193804.1; AB 240155.1) as the outgroup. Since the phylogenetic placement of *Idiosepiidae* within the cephalopods is not clarified (see 'Introduction'),

Table 2. PCR protocols for the 4 gene loci; for 16S rRNA, 2 different protocols were used

	12S rRNA		16S rRNA				COI and COIII	
Initial denaturation	2 min	94°C	2 min	94°C	2 min	94°C	2 min	94°C
Denaturation	25 s	94°C	20 s	94°C	20 s	94°C	2 min	94°C
Annealing	30 s	46°C	20 s	50°C	20 s	43°C	10 s	42–45°C <sup>a</sup>
Elongation	40 s	72°C	40 s	72°C	40 s	72°C	1 min 45 s	72°C
	} 35 cycles		} 15 cycles		} 20 cycles		} 36 cycles	

<sup>a</sup>With a temperature gradient of 1.8°C

it made no difference whether we used Sepiidae or Teuthidae species as the outgroup because the relationship within *Idiosepius* remains constant.

Maximum parsimony (MP) analyses of the aligned data with unordered and equally weighted characters, 100 random addition replicates and tree bisection and reconnection (TBR) branch swapping were conducted in PAUP (4.0b10) (Swofford 2002). A maximum of 100 most parsimonious trees were saved for each replicate, and the resulting trees subsequently submitted to another round of TBR branch swapping. Branch support was assessed by bootstrapping (1000 replicates) using the heuristic search option and TBR branch swapping.

Maximum likelihood (ML) searches of the individual genes were started with the neighbor-joining (NJ) tree and subsequent TBR branch swapping, using the model and parameters returned by the Akaike Information Criterion (AIC) in MODELTEST 3.06 (Posada & Crandall 1998). A Bayesian analysis with MR-BAYES 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) was performed on the data, with individual partitions for each gene. Individual MODELTEST runs for each data partition returned the GTR+ $\Gamma$ +I model for COI and 16S, which we also applied to the COIII data (TIM+ $\Gamma$ +I suggested by AIC). The Kimura81 model with unequal base frequencies + $\Gamma$ +I was suggested for the 12S partition. The chains were run for 5 million generations, a sampling frequency of 100 and a burn-in of 5000 after visual assessment of chain convergence.

*Idiosepius notoides* appeared as a monophyletic sister group to the other members of the genus in all analyses. We therefore selected this clade as the outgroup instead of *Sepia* and ran the above analyses again to avoid long branch attraction artefacts due to the long branches of *Sepia*. Eliminating identical sequences from the data set resulted in 46 unique *Idiosepius* sequences.

## RESULTS

Overall, the analyses of the 4 mitochondrial loci were suitable to differentiate between the species and population level for *Idiosepius*. The phylogenetic analyses of the partial 12S rRNA, 16S rRNA, COI and COIII genes clearly revealed that *I. notoides*, *I. pygmaeus* and *I. picteti* were monophyletic (all analyses), whereas *I. biserialis* and *I. paradoxus* were polyphyletic for the same analysis.

Similar tree topologies (Fig. 2) resulted from the combined phylogenetic analyses of all 4 gene loci

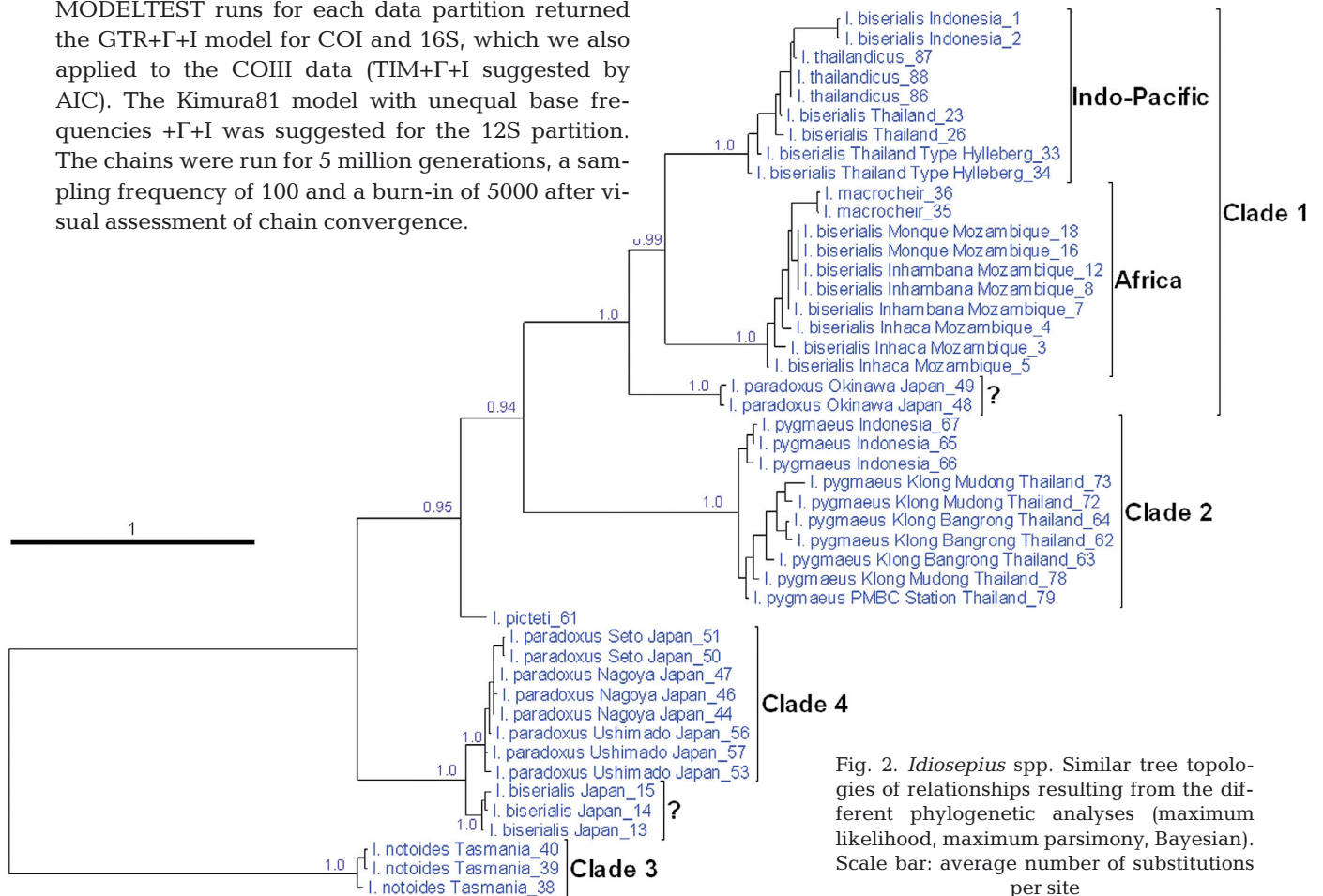


Fig. 2. *Idiosepius* spp. Similar tree topologies of relationships resulting from the different phylogenetic analyses (maximum likelihood, maximum parsimony, Bayesian). Scale bar: average number of substitutions per site

using NJ, MP, ML and Bayesian inference. The MP analysis of the combined dataset was stopped at 1.5 million most parsimonious trees (tree length 888, consistency index [CI] 0.6036, rescaled consistency index [RC] 0.575). The strict consensus tree showed polytomies within the monophyletic species clades only. Genetic distances between members of different clades ranged from 5 to 13%, whereas distances within Clades 2 to 4 did not exceed 2%. The greatest intra-clade distances of up to 5% occurred in Clade 1 between Indian Ocean and Pacific individuals of *Idiosepius biserialis*.

Four well-supported clades were consistently recovered. The 2-rowed species *Idiosepius biserialis* (Clade 1) was robustly subdivided into 2 populations: the African group consisting of *I. biserialis* from the different locations in Mozambique, and the Indo-Pacific group comprising all *I. biserialis* specimens from Thailand and Indonesia. *I. macrocheir* nested within the African group of *I. biserialis*, whereas *I. thailandicus* nested within the Indo-Pacific group. Specimens of *I. paradoxus* from Okinawa Island (Japan)

formed a sister group to *I. thailandicus*, *I. macrocheir* and *I. biserialis* (African and Indo-Pacific group). The 4-rowed group was represented by *I. pygmaeus* (Clade 2) and *I. notooides* (Clade 3). Clade 4 comprised specimens of *I. paradoxus* from different locations (Nagoya, Ushimado and Seto Island, Japan) as well as individuals of *I. biserialis* from Japan. The position of the type specimen of *I. picteti* was unstable and varied with markers and analysis methods (Fig. 3).

Comparison of the Bayesian data of each gene locus indicated divergences between the *Idiosepius biserialis* populations. In the 16S and COIII analyses, the 4 clades were well represented (Fig. S1A,B in the supplement), while in the 12S and COI analyses, the genus was geographically divided: Africa (with *I. biserialis* from Africa and *I. macrocheir*), Indo-Pacific (comprising *I. biserialis* Indo-Pacific, *I. pygmaeus*, *I. paradoxus* Okinawa, *I. picteti* and *I. thailandicus*), Japan (*I. biserialis* from Japan and *I. paradoxus*) and the Tasmanian group (*I. notooides*; Fig. S1C,D in the supplement).

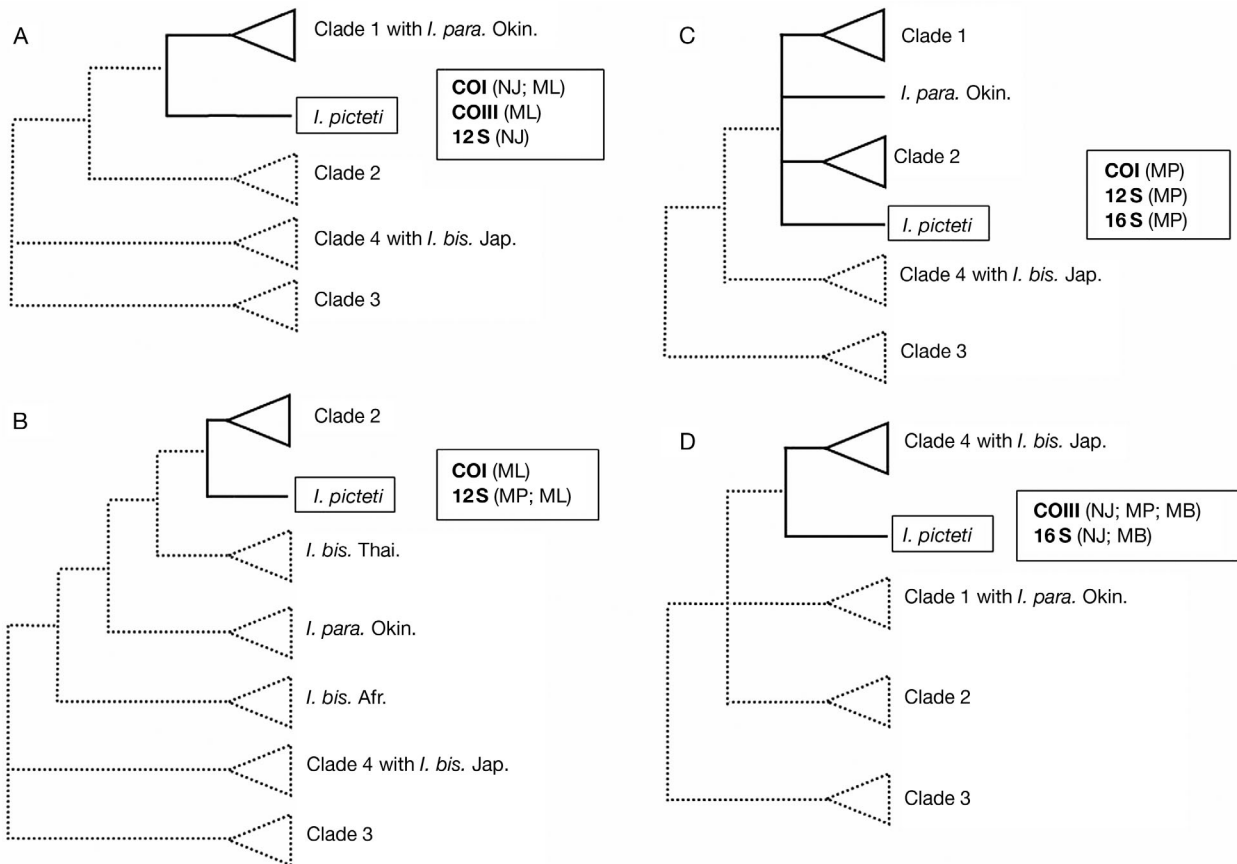


Fig. 3. *Idiosepius picteti*. (A–D) Cladograms representing its systematic position in the phylogenetic analysis using all 4 gene loci and neighbour joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian (MB) inference. *I. para.*: *I. paradoxus*; *I. bis.*: *I. biserialis*; Okin.: Okinawa; Jap.: Japan; Thai.: Thailand; Afr.: Africa



Characterisation of COI and COIII in the African population of *Idiosepius biserialis* indicated a slight divergence for *I. macrocheir*, which was not observed using 12S and 16S rRNA genes (Fig. S1B,D in the supplement).

## DISCUSSION

Since the earlier taxonomic study by von Byern & Klepal (2010) raised questions about the validity of *Idiosepius thailandicus* and *I. macrocheir*, the aim of our study was to re-evaluate the phylogenetic relationships among all nominal *Idiosepius* species.

### *Idiosepius thailandicus*

As hypothesized earlier (Nabhitabhata & Suwanmala 2008, von Byern & Klepal 2010), the systematic position of *I. thailandicus* is uncertain. Based on the taxonomic characters proposed by Nesis (1987) and von Byern & Klepal (2010), it is impossible to distinguish *I. biserialis* and *I. thailandicus*. Moreover, cross-mating experiments have shown that both species mate with each other and produce fertilized eggs (Nabhitabhata & Suwanmala 2008), whereas *I. biserialis* and *I. pygmaeus* do not show mutual mating behaviour.

The present phylogenetic data confirm that *Idiosepius thailandicus* is robustly nested within the Indo-Pacific group of *I. biserialis* and support the hypothesis that *I. thailandicus* represents a junior synonym of *I. biserialis*. However, both species are differently distributed geographically along the coasts of Thailand: *I. thailandicus* in the Gulf of Thailand and *I. biserialis* in the Andaman Sea. This raises the question whether the 2 species are able to come together in the same area. Moreover, it is doubtful whether both species occupy the same habitat, and the habitat preference of *I. thailandicus* is uncertain. While we found the species exclusively near mangrove roots in the eastern part of Thailand (Chontaburi province), the type material was collected by Chotiyaputta et al. (1991) with a push net from 2 to 5 m in the estuary of the Donsak River in the southern province of Surat Thani. The depth and collection method point to a sea grass bed habitat, but the original authors could not be contacted to clarify this point. Moreover, it cannot be excluded that the 'mangrove' specimens we found in Chontaburi province are individuals that drifted into this habitat; we never controlled the estuary for possible sea grass beds.

The situation is clearer for *I. biserialis*: all specimens were collected exclusively in sea grass beds (Suwanmala et al. 2006).

Finally, the geographical distribution and habitat preference of both species remain poorly known and will require further investigation. Nonetheless, our study as well as the taxonomic characterisation by von Byern & Klepal (2010) and cross-mating experiments by Nabhitabhata & Suwanmala (2008) strongly indicate that *Idiosepius thailandicus* was incorrectly characterised as a separate species and actually represents a junior synonym of *I. biserialis*.

### *Idiosepius macrocheir*

Although Voss (1962) characterised *I. macrocheir* as a 4-rowed species, morphological re-evaluations of this systematic character (Nürnberger et al. 2006, von Byern & Klepal 2010) as well as our present data clearly indicate that *I. macrocheir* is related to the 2-rowed instead of the 4-rowed group. Additionally, since Voss (1962), no further individuals with a 4-rowed arrangement of suckers on the tentacular clubs have been found in Mozambican waters, although several collection expeditions by the South African Museum in this region have been undertaken in the past. Moreover, the paratypes SAM 185 & 186 of *I. macrocheir* were collected in the same area and habitat where we recently caught specimens of *I. biserialis* for this research. Our molecular phylogenetic analysis as well as the morphological data provide strong evidence that *I. macrocheir* resembles *I. biserialis* and may also be a junior synonym, as proposed for *I. thailandicus*.

### *Idiosepius picteti*

In addition to the uncertain phylogenetic relationships of *I. thailandicus* and *I. macrocheir*, the systematic position of *I. picteti* is doubtful. There is evidence that the handling and fixation of the holotype sample not only led to a mis-arrangement of the suckers on the tentacular clubs but also considerably changed its body form. A non-anaesthetised specimen of *I. pygmaeus* (NHMW no.103225) collected in Indonesia became deformed due to fixation (von Byern & Klepal 2007) and showed the same shape as the holotype specimen of *I. picteti* (Joubin 1894). Apart from this coincident similarity, *I. picteti* showed close congruence to *I. pygmaeus* in the shape and number of suckers on the hectocotylised arms (von Byern &

Klepal 2010). This led to the hypothesis that *I. picteti* was likewise incorrectly classified as a single species and rather resembles a subspecies of *I. pygmaeus*, as already described for *I. pygmaeus hebereri* (Grimpe 1931).

Our phylogenetic analyses failed to provide a clear relationship of *Idiosepius picteti* to *I. pygmaeus* or any other *Idiosepius* species, as shown in Fig. 2. According to the different phylogenetic analyses of the 4 gene loci, the phylogenetic position of *I. picteti* remains uncertain because the relationship with the 2-rowed group contrasts with the morphological data. The reason for the incorrect allocation may reflect damage of the 4 gene loci caused by the long storage and/or fixation. Despite 5 amplification and sequencing runs, the data and analysis remained the same. More material or other gene loci need to be analysed to verify with certainty the systematic status of *I. picteti*.

### ***Idiosepius biserialis***

Our molecular phylogenetic analyses clearly indicated genetic variability coinciding with geographic isolation among the *I. biserialis* populations sampled. This variability, however, could not be determined based on taxonomic characters (von Byern & Klepal 2010) or habitat preferences (see Table S1 in the supplement). Specimens of *I. biserialis* from Japan were morphologically classified to the 2-rowed group (von Byern & Klepal 2010) and appeared to be closely related to the 4-rowed species *I. paradoxus*. This phylogenetic distinctiveness compared to individuals from Africa and the Indo-Pacific cannot be attributed to geographic diversification, because *I. biserialis* from Japan should then still be closer to the individuals of Clade 1. We currently have no explanation for this phylogenetic distinctiveness. One obvious habitat difference of *I. biserialis* in Japan from that of the African and Indo-Pacific population is water temperature. The Japanese population remains in cool temperature zones (water temperature  $\leq 15^{\circ}\text{C}$ ), while the African and Indo-Pacific specimens occur in tropical waters ( $\geq 25^{\circ}\text{C}$ ). Temperature seems to be responsible only for species migration, but it could also potentially explain local adaptation and future speciation. Unfortunately, the *I. biserialis* samples collected in Japan were occasional findings; the precise area and habitat in which the animals normally occur remain unknown. More individuals are needed to verify their taxonomic characters and indicate their relationship to *I. biserialis* and/or *I. paradoxus*.

### ***Idiosepius paradoxus*, Okinawa Island**

A situation similar to that of *I. biserialis* from Japan exists for *I. paradoxus* from Okinawa Island. Based on the geographical assignment of Okinawa Island to Japan, these individuals were defined as *I. paradoxus*. Based on the number of suckers on the tentacular clubs, which seems to be the only possible way to taxonomically differentiate the species (von Byern & Klepal 2010), the individuals from Okinawa Island (average  $40 \pm 6$  suckers) were more closely related to the 2- ( $\leq 46$  suckers) than to the 4-rowed species ( $\leq 83$  suckers). The investigated animals, however, were exclusively females, precluding a taxonomic classification based on the hectocotylysed fourth arm pair as proposed by the systematic key of Nesis (1987).

The current phylogenetic analyses also indicate a closer relationship of Okinawa Island *Idiosepius paradoxus* to *I. biserialis* than to *I. paradoxus* from mainland Japan. In our opinion, this mistake was made solely because of the geographical assignment of Okinawa Island to Japan. A larger sample collection of this population is planned in order to provide more information on their taxonomic characters and to classify the specimen from Okinawa Island clearly as *I. biserialis* or *I. paradoxus*.

### **Geographical distribution and dispersal**

It remains fascinating how these animals—miniature in size, extremely short-lived ( $\pm 150$  d; Jackson 1988, Sato et al. 2008) and with a relatively strict inshore habitat preference (see Table S1 in the supplement)—could exhibit such a huge population range. We know from incidental observations that water temperature rather than geographical barriers or habitat preferences seem to affect animal migration/limitation. Populations of *Idiosepius pygmaeus* occur in the northern and north-eastern part of Australia, whereas *I. notoides* exclusively inhabit the southern part of Australia and Tasmania. The records show that these 2 species never overlap but are strictly separated by the warmer current off the coast of Queensland (Norman & Reid 2000). Seasonal observations by Sato et al. (2009) confirmed that individuals of *I. paradoxus* are transported with the warm water to the north of Japan, but are not able to survive the cold winter there. Unexpectedly, *I. paradoxus* was observed in Russian waters (Nesis et al. 2002b) due to the warming of surface waters in Peter the Great Bay. This indicates that the animals can move 1000 km, e.g. though the Japan Sea, up to Rus-

sia within less than 6 mo. Nonetheless, this migration distance is small compared to the distance which the smallest species, *I. biserialis*, overcame: from Africa through the Indo-Pacific to Japan.

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