Università degli Studi di Roma "La Sapienza"

Facoltà di Scienze Matematiche, Fisiche e Naturali

Corso di Dottorato di ricerca in Scienze Ecologiche

Titolo della tesi:

Mudskippers (Gobiidae: Oxudercinae): ecology and evolution along the water edge, and possible use as a biomonitor of tropical intertidal areas

Mudskipper fight at sunset (*Boleophthalmus dussumieri*, Bandar Abbas, Iran, 2006); photo: G. Polgar

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Abstract

Mudskippers (Gobiidae: Oxudercinae) are highly amphibious and closely related tropical gobies. Systematic, anatomical and physiological studies suggest that they increasingly adapted to semi-terrestrial life throughout their evolution. Their physiological adaptations match ecological definitions based on environmental conditions of their habitats. Observations also suggest that synecological factors may be key selective agents. In this respect, recent palaeoecological and palaeontological findings suggest that mudskippers may help to understand some of the factors and processes that defined the scenario of the Devonian vertebrate eco-evolutionary transition.

Mudskippers are also proposed as a biomonitor of the health and integrity of highly endangered mangrove and tropical tidal flat ecosystems, as suggested by their differential distribution along the intertidal zone. This also identifies them as possible flag species.

Introduction

The oxudercine gobies (Teleostei: Gobiidae: Oxudercinae) include 39 species in 10 genera. Seven genera and 27 species, known as ‘mudskippers’, are “fully terrestrial for some portion of the daily cycle” (Murdy, 1989). Mudskippers and their close relatives are distributed along the Atlantic coasts of Africa and in the whole Indo-West-Pacific region (Murdy, 1989). These species are closely linked to tropical intertidal ecosystems, where they colonised several peritidal soft-bottomed habitats, from the low intertidal to the high supratidal zone. More recently, they were also found in the lower tracts of tropical rivers (Khaironizam & Norma-Rashid, 2003; Polgar & Sacchetti, under review).

The aims of this study are: 1) to contribute to the habitat ecology of mudskippers, with respect to their different degrees of adaptation to terrestriality, and renew the scientific interest in this group as a vertebrate model for the eco-evolutionary transition from water to land (Polgar & Crosa, accepted; Polgar et al., accepted; Polgar & Sacchetti, under review); and 2) to propose mudskippers as possible biomonitors for the health assessment and management of tropical intertidal ecosystems (Polgar, 2008a). During this project, I published and regularly updated a scientific website on mudskippers (Polgar, 2008b); I described the habitat and distribution of several species of mudskippers from the two biodiversity hotspots for oxudercine gobies: Peninsular Malaysia (Polgar, 2008a; Polgar & Crosa, accepted) and Papua New Guinea (Polgar & Sacchetti, under review); and I made a survey in southern Iran, where I studied peculiar host-parasite interactions between a marine leech and a mudskipper (Polgar *et al.*, accepted). Other projects are currently being followed in collaboration with the University “La Sapienza”, Rome (Dr. V. Bartolino); the University “Ca’ Foscari”, Venice (Prof. P. Torricelli, Dr. S. Malavasi); the University of Padua, Padua (Prof. T. Patarnello, Dr. L. Zane); the University of Insubria, Varese (Prof. L. Crosa); the University of “Milano Bicocca”, Milan (Prof. P. Galli, Dr. G. Strona); the Natural History Museum, London (Dr. L. Rüber); and Prof. J.A. Clack (University Museum of Zoology, Cambridge). Several publications are currently in preparation.

Mudskippers as an eco-evolutionary model for the sea-to-land transition

The first documented naturalistic observations on mudskippers date back to the seventeenth century. Two of the eight Linnean goby species (presently > 200 genera and 1,800 species) are mudskippers: *Boleophthalmus pectinirostris* (Linnaeus, 1758); and *Periophthalmus barbarus* (Linnaeus, 1758). During the eighteenth century the first European explorers and tropical ichthyologists captured and described several other genera and species (e.g. Bleeker, 1874; Cantor, 1849; Cuvier & Valenciénnes, 1837; Lesson, 1830; Hamilton, 1822; Bloch & Schneider, 1801).

Then Darwin presented his theory on the origin of species. In the first half of the nineteenth century up to the first half of the 60s, anatomical, morphological and taxonomic studies of mudskippers (e.g. Baumeister, 1913; Lele & Kulkarni, 1938; Eggert, 1929) were prompted by vivid evolutionary interest, with particular reference to the origin and evolution of vertebrate terrestriality. This interest was certainly bolstered by the discovery of the fossil remains of the first piscine prototetrapod *Ichthyostega* by Säve-Söderberg and Jarvik, in 1929-1931 (Jarvik, 1996).

In the ‘40s-‘50s the neodarwinian synthesis was born, and in the following years mudskippers continued to be intensively studied, even though researches were hampered by considerable systematic confusion and harsh operational difficulties in the field (e.g. Harris, 1961; Stebbins & Kalk, 1961; Van Dijk, 1959).

Suddenly, and with few exceptions (e.g. Gordon *et al.*, 1969), in the second half of the ‘60s the ecological and evolutionary interest on mudskippers as possible model for the vertebrate transition sharply decreased. This interest was almost completely directed to freshwater air-breathing fishes and ceratodontiform sarcopterygians, as a consequence of the well known theories of A.S. Romer on tetrapod evolution (e.g. Romer, 1972; 1967). The palaeoenvironmental scenario depicted by Romer for the vertebrate water-to-land transition was of tropical, continental, closed water bodies, seasonally and chronically affected by water shortages. This scenario is completely different from the intertidal mudflats where mudskippers occur. Romer’s theory implied that bimodal breathing and aquatic respiration in hypoxic and hypercapnic waters were crucial aspects of the vertebrate transition. Mudskippers, relatively less derived air-breathers and living in marine open systems, were disregarded as possible models, and considered as an evolutionary ‘dead-end’ pathway to terrestriality, a hyper-specialised group, raising bare naturalistic curiosity. Freshwater air-breathing fishes, including dipnoan sarcopterygians, with their complex adaptations to hypoxia and chronic water shortages, were much more similar to Romer’s model.

Numerous ecological and physiological studies were then conducted on such species in seasonal and tropical freshwater habitats. In the ‘70s and ‘80s, fish physiologists, thanks also to a considerable investment in this research area by the NSF, offered a significant contribution to the general understanding of vertebrate respiration physiology, with profound effects also on biomedical applications (Graham, 1997). In this period, numerous studies were made on mudskippers’ anatomy, physiology and histology, mainly on air breathing, but also on their osmoregulation and excretion (e.g. Iwata *et al.*, 1981; El-Sayed & Safer, 1985; Al-Kadhomiy & Hughes, 1988).

In 1989 Murdy published his morphological systematic revision of the Oxudercinae (one of the few ones in the whole suborder Gobioidei). Murdy greatly simplified their taxonomy by pruning out all invalid synonymies, and lumping several taxa at subspecific and specific level.

In the ‘90s the comparative physiology of mudskippers was still a fertile scientific area (El-Sayed & Safer, 1992; Chew & Ip, 1992; Ishimatsu *et al.*, 1999), and this trend is still very productive until recently (e.g. Sakamoto *et al.*, 2002; Ip *et al.*, 2005; Gonzales et al., 2006).

Very few investigations in the field had ever been made, most being semi-quantitative, descriptive, or anecdotic accounts (e.g. Stebbins & Kalk, 1961; Sarker *et al.*, 1980; Nursall, 1981). The eco-ethological interest grew in the ‘80s, ‘90s and up to date, when more quantitative studies were made (e.g. Clayton & Vaughan, 1988; Colombini *et al.*, 1995; Swennen *et al.*, 1995; Ikebe & Oishi, 1997; Ishimatsu *et al.*, 1998; Takita *et al.*, 1999; Ishimatsu *et al.*, 2000; Baeck et al., 2007). Several recent new records and species descriptions probably witness the growing penetration of man into mangrove systems, previously largely unexplored (Lee et al., 1995; Murdy & Takita, 1999; Khaironizam & Norma-Rashid (2002; 2003); Darumas & Tantichodok, 2002; Larson & Takita, 2004; Jaafar et al., 2006; Wang et al., 2006; Jafaar & Larson, 2008; Polgar & Khaironizam, in press). After 2000 some few experimental studies were also made in the field (e.g. Ishimatsu *et al.*, 2007).

During the last ten years, important paleontological and palaeoenvironmental findings strongly question the Romer's paradigm, showing a long and complex semi-aquatic evolutionary history of Devonian tetrapodomophs and prototetrapods (Shubin *et al.*, 2006; Daeschler *et al.*, 2006; Ahlberg & Clack, 2006; Boisvert, 2005; Clack, 2005; 2004; 2002; Long & Gordon, 2004; Coates *et al.*, 2002). According to these recent findings, the Devonian vertebrate transition seemingly took place for several millions of years in tropical semi-terrestrial and open aquatic systems, such as alluvial plains and intertidal flats, were the ancestors of all extant tetrapods evolved anatomical complexes of keystone characters, such as the tetrapod limb. Biotic selective factors (i.e. synecological interactions) also gradually appeared to be as much as, or even more important as abiotic factors (Sayer, 2005; McNamara & Selden, 1993; Sayer & Davenport, 1991; Liem, 1987). In particular, these data support previous hypotheses that 1) air-breathing adaptations initially evolved to *remain* in water in extreme abiotic conditions, and 2) the transition should have occurred in *continuously* wet conditions (Inger, 1957; Liem, 1987). This suggests that intertidal areas, where mudskippers live, are the best candidates for a model of the paleoenvironmental scenario of the initial phases of this evolutionary process, during the first diversification of the tetrapod stem group (Clack 2007; Daeschler *et al.*, 2006).

Some scientists still follow Romer's hypothesis, disregarding mudskippers as possible convergent models for the vertebrate eco-evolutionary transition (e.g. Graham & Lee, 2004). They rather focus on the selective pressures exerted by stringent environmental factors on physiological processes such as air-breathing, extensively evolved in closed, freshwater aquatic systems. Nonetheless, they continued to work intensively on mudskippers (e.g. Lee *et al.*, 2005).

Schultze (1999) presented evidence that the intertidal environment was the probable habitat of Givetian-Frasnian prototetrapods, and showed seemingly convergent morphological adaptations between mudskippers and these fossils. During my studies, the habitat distribution of mudskippers showed a gradual development of adaptations to semi-terrestrial conditions, including the colonisation of freshwater swamps and the lower tracts of rivers (Polgar & Crosa, accepted; Polgar

& Sacchetti, under review). Other findings (Polgar *et al.*, accepted) even suggest the presence of host-parasite ecological dynamics which are strikingly similar to those found in some salamanders (Goater, 2000).

These findings suggest that this group may indeed be the only living example of a vertebrate independent evolutionary path from water to land, facing ecological selective pressures similar to those ones experienced by our tetrapod ancestors. The understanding of how mudskippers progressively did gain and are still ‘gaining ground’ (Clack, 2002), may concretely assist in hypothesis building in the attempt to reconstruct paleontological, palaeoenvironmental and evolutionary scenarios of one of the conundrums of vertebrate evolution.

Mudskippers as biomonitors for Indo-West-Pacific mangrove and mudflat ecosystems

From an eco-evolutionary standpoint, mangroves are a peculiar and relatively not diverse polyphyletic group, including only 65 species and 9 orders, which successfully colonised tropical intertidal habitats, at least from the Late Cretaceous up to the present (Ellison *et al.*, 1999; Tomlinson, 1986). Ecological studies, pioneered by W.E. Odum (e.g. Odum & Heald, 1975) showed that mangrove systems can be locally highly productive (Sasekumar, 1980; Clough, 1992), and present open nutrient cycles with relatively fast turnover rates and high P/R ratios, typical of young forests (Hogarth, 1999). These characteristics, determined by the peculiar adaptations of mangrove plants to cope with extremely dynamic environmental conditions (Woodroffe, 1992), make them highly resilient and robust relative to other tropical forest ecosystems (Ellison, 2002; Hogarth, 1999).

Nonetheless, a dramatic reduction of mangrove vegetation coverage occurred worldwide in the last 40-50 years (Wilkie & Fortuna, 2003; Por & Dor, 1984). In the ‘60s mangrove forests fringed about 60-75% of tropical coastlines. Almost 25% of the total coverage got lost in the last 20-30 years, with average destruction rates of 1-2% per year, and peaks of 8% (Wilkie & Fortuna, 2003; A. Quarto, unpubl. data). These figures make mangrove forests some of the most endangered ecosystems on earth.

Anthropogenic impact mostly results in habitat destruction caused by demographic growth along coast; agriculture and industrial coastal development; conflicts in multiple use of coastal resources; and overexploitation. Pollution, mainly agriculture and industrial wastes, oil spills, and wave action increased by boat traffic, is also degrading mangroves both from the land and the sea (e.g. Sasekumar, 1974; 1980), while more recently the big business of shrimp farming, especially in South East Asia and Indonesia, greatly contributed to habitat destruction (e.g. Wilkie & Fortuna, 2003; Menasveta, 1996; Lee, 1992).

Until recently, mangrove ecosystems, or mangals (Macnae, 1968) were considered as almost impenetrable and unproductive wastelands, harbouring swarms of hematophagous insects (Ceratopogonidae, Culicidae), vectors of deadly pathogens (e.g. Dengue viruses, *Plasmodium* spp., yellow fever viruses), and other dangerous animals (e.g. crocodiles, poisonous snakes and fishes). Instead, several studies showed that mangroves have both considerable economic and non-economic values, impacting both marine and terrestrial ecosystems (e.g. Hogarth, 1999). In particular, though still subject to scientific scrutiny (Sheridan & Hays, 2003), it is largely accepted that mangrove systems act as nurseries for the larval stages of several fish and invertebrate neritic and pelagic species of commercial importance (e.g. Laegdsgaard & Johnson, 2001; Chong *et al.*, 1996; Sasekumar *et al.*, 1992; Macnae, 1968). In an olistic perspective, their impact on land and adjacent marine systems, such as coral reefs and sea grass beds was put forward in the last years (Wolanski, 2001). Mangrove systems were shown to act as nutrient sinks and sedimentary traps for the suspended load discharged by rivers, and limit coastal erosion (Duke & Wolanski, 2001). More recently, the tsunami disasters of 2005 put also in evidence the value of mangrove forests as buffer zones between sea and land, mitigating the action of tropical storms, or even exceptionally energetic events (Danielsen *et al.*, 2005; Mazda *et al.*, 1997). Other economic values include industrial timber and charcoal production, traditional products, local subsistence of traditional cultures, and more recently, ecotourism (Ellison, 2000; Hogarth, 1999).

Tropical tidal flats are even less known, more difficult to study (Raffaelli & Hawkins, 1999; Holme & McIntyre, 1984), and maybe more impacted by pollution from the sea. Nonetheless, their ecological role and impact on fisheries and adjacent ecosystems is possibly as much important, especially when associated with mangrove systems, as several studies suggest (Dittmann, 2002; Chong *et al.*, 1996; Butler *et al.*, 1997; Sasekumar & Chong, 1986).

Mudskippers are closely linked to such systems, being differentially distributed along the whole intertidal zone (Polgar, 2008a; Polgar & Crosa, accepted). For their abundance, benthic and burrowing habits, and considerable resistance to highly polluted conditions, mudskippers were frequently used in ecotoxicological studies. Since mudflats are efficient traps of fine sediments and sinks of chemical compounds, these fishes can absorb and concentrate many different pollutants, from heavy metals and other chemicals present in industrial effluents (e.g. Eboh *et al.*, 2006; Chhaya *et al.*, 1997; Kundu *et al.*, 1995; Everaarts *et al.*, 1994), to pesticides (Islam *et al.*, 2006), fluoride compounds (Shaikh & Hiradhar, 1988), and hydrocarbons (Nakata *et al.*, 2002). Mudskippers are either consumed or used as baits in many regions, such as Middle East, India, South East Asia, China, Taiwan, southern Japan and Australia (Kizhakudan & Shoba, 2005; Bucholtz & Meilvang, 2005; Zhang & Hong, 2003; Clayton, 1993; Zhang *et al.*, 1989; *pers. obs.*; T. Takita, *pers. comm.*). But even where not directly used, they are locally very abundant, being important prey items for many marine transient species of fish and crabs, also of commercial importance, and of several reptiles and birds (Clayton, 1993; Jayne et al., 1988; *pers. obs.*). Therefore, the potential for bioaccumulation and health issues wherever mudskippers occur and are directly or indirectly consumed is reasonably substantial.

Furthermore, the diverse sympatric assemblages of mudskipper species often present in several of these areas, would allow to make diversified analyses at different levels of the intertidal zone, since our studies indicate specific patterns of habitat differentiation (Polgar & Crosa, accepted; Polgar & Sacchetti under revision; Muhammad Ali & Norma-Rashid, 2005; Takita *et al.*, 1999). Such ecological partition also show that mudskippers can not only be used to monitor the effects of pollution, but also the effects of habitat destruction, both from the land and from the sea (Polgar, 2008a). As a consequence, mudskippers can also be used as bioindicators of the state of health and integrity of mangrove and mudflat ecosystems.

Results and discussion

Habitat differentiation and evolutionary ecology

Both studies made on the habitat water availability of mudskippers demonstrated clear habitat differentiation among species. Malayan mudskippers (Polgar & Crosa, accepted) can be grouped into three different guilds with different levels of terrestriality. This pattern is mirrored by their specific physiological adaptations to terrestriality, while some differences with the morphological systematic relationships were found. My study of the mudskippers from Papua New Guinea (Polgar & Sacchetti, under review) depicts a more complex scenario, which nonetheless is compatible with a sequential evolutionary origin of eco-physiological and eco-ethological adaptations to more terrestrial conditions among genera. In particular, within the monophyletic tribe Periophthalmini, Murdy's cladogram (1989) describes a sequential cladogenesis of the genera *Zappa*, *Scartelaos*, *Boleophthalmus*, *Periophthalmodon* and *Periophthalmus*. Assuming that the absence of amphibious adaptations is plesiomorphic for this group, it would be expected that less derived genera include more aquatic species, which also generally resulted from our analyses, with few exceptions. Instead, more phylogenetically derived species, included in the sister genera *Periophthalmus* and *Periophthalmodon*, present a wide array of habitats and adaptations, from relatively aquatic to extremely terrestrial lifestyles (Polgar & Sacchetti, under review). Such results suggest the presence of several parallel evolutionary pathways to terrestriality within genera.

Though only a limited number of species was used for physiological studies in the laboratory, physiological and anatomical traits of mudskipper congeneric species are mirrored by the degree of their terrestriality, inferred by their habitat distribution.

Nonetheless, two considerations should be made.

1) The present systematic and phylogenetic hypothesis (Murdy, 1989), is based on a cladistic analysis of morphological *and* eco-ethological characters. Consequently, this hypothesis depends also on the different degrees of adaptation to semi-terrestrial conditions of mudskipper species. Therefore, to say that the correspondence between phylogeny and habitat differentiation demonstrates that improved adaptations to semi-terrestrial conditions evolved at each cladogenetic event, would be at risk of circular reasoning.

2) More recent molecular findings seem to question Murdy's hypothesis (Thacker, 2003; Akihito, 2000), suggesting a close relationship between some members of the Oxudercinae and some species of the Amblyopinae, suggesting that these subfamilies are actually paraphyletic.

I coded Murdy's matrix based on his descriptions, I made a MP phylogenetic analysis (PAUP v. 4.0), and found the same cladogram topology (Polgar & Crosa, accepted: ESM, S1). When I eliminated the eco-ethological characters related to amphibious lifestyles and air-breathing, no topological changes occurred within the tribe Periophthalmini, except for a strong polytomy at the first node of this clade (*unpubl. data*). As a consequence, except for the relationship between *Zappa*, *Pseudapocryptes*, and *Apocryptes* and the other genera of Periophthalmini, it actually exists independent morphological evidence of a correspondence between systematic patterns and environmental aquaticity for all other genera of Periophthalmini (i.e. *Scartelaos*, *Boleophthalmus*, *Periophthalmodon* and *Periophthalmus*).

Of course, if future molecular studies will show that Oxudercinae is paraphyletic, our eco-evolutionary considerations would be probably affected. On the other hand, the putative close relationship with amblyopine gobies, which also present air-breathing behaviours and adaptations (Gonzales *et al.*, 2006; Graham, 1997), even if more aquatic, extends the possibilities to explore the evolution of amphibious lifestyles and the colonisation of semi-terrestrial habitats from more aquatic ecological conditions.

The taxonomy and systematics of Gobioidei had always been problematic (e.g. Akihito *et al.*, 2000). In particular, while morphological discrimination at the genus level is not particularly difficult, suprageneric and infrageneric taxa are highly unstable, with the only exception of suborder and family levels. This is probably a consequence of both the small size of gobies, and their recurrent heterochronic evolution (e.g. paedogenesis, neoteny), which is seemingly widespread among these species. No complete molecular phylogeny at the genus level is actually available for the Oxudercinae and Amblyopinae.

However, the differential habitat use and the wide range of environmental conditions where mudskippers were found (Polgar & Crosa, accepted; Polgar & Sacchetti, under review), together with the presence of mudskipper parasites limited to specific habitat conditions (Polgar *et al.*, accepted), prompt for extensive comparative studies of their adaptations to terrestriality. This wide array of adaptations also further extends the possibility to use these species as convergent models to understand the selective pressures and conditions experienced by transitional vertebrates evolved in analogous ecological and evolutionary contexts, both living and extinct.

In particular, single species were found in a wide range of habitat types and salinity conditions, both in deltas and in the lower tracts of large rivers (Polgar & Sacchetti, under review). These observations would suggest the insubstantial nature of the age-old diatribe of the freshwater vs. marine origin of terrestrality in Devonian prototetrapods (e.g. Schultze, 1999; Graham, 1997).

Future developments may include: molecular phylogenies and systematic revisions; quantitative studies of their spatial ecology; phylogeographic studies and biogeographic revisions; comparative analyses of convergent morphological characters in mudskippers and extinct prototetrapods; and eco-ethological researches. We also believe that a deeper understanding of their ecology and evolution would be achieved through the experimental study of synecological interactions between mudskipper sympatric species (at inter- *and* intraspecific level), their preys and predators (McNamara & Selden, 1993; Sayer & Davenport, 1991; Liem, 1987).

Applied ecology and environmental management

The SAR found in Malayan mudskippers (Polgar 2008a) and their apparent zonation indicate the possible use of these species as bioindicators of the state of health and integrity of mangrove and mudflat ecosystems both from the land and from the sea. This would be particularly useful for tropical coastal ecosystem management and conservation, since habitat destruction is usually starting from land, while pollution is mainly acting from the sea, resulting in a differential anthropogenic impact along the intertidal zone. As a result, some mudskipper species living at higher levels may be already endangered: their populations being fragmented, or locally extinct (e.g. *Periophthalmodon septemradiatus*: Polgar, 2008a). On the contrary, species living on mudflats are more intensively and chronically affected by contamination of several pollutants. The close linkage of some mudskipper species to lower intertidal levels indicate that analyses of contaminants' concentrations in the tissues of these species should be standardised and routinely measured during pollution assessments in tropical intertidal areas (Kruitwagen *et al.*, 2006).

Our observations in Papua New Guinea (Polgar & Sacchetti, under review) and Peninsular Malaysia, where aboriginal cultures (Wapi village; Orang Laut) regularly consume mudskippers (pers. obs.), confirm previous accounts of human use. Contact between mudskippers and humans is facilitated by the considerable resistance of these fish to organic pollution (e.g. Takita *et al.*, 1999; *pers. obs.*); their resistance to toxic compounds, probably related with air-breathing and their detoxifying physiological adaptations, is also one of the reasons for their frequent use in eco-toxicological studies (e.g. Islam *et al.*, 2006; Nakata *et al.*, 2002; Chhaya *et al.*, 1997; Shaikh & Hiradhar, 1988). Therefore, it follows that bioaccumulation and health issues are worth to be investigated in human settlements located nearby sources of pollution, wherever local populations make use of these species.

Finally, mudskippers would also be excellent flag species (Primack, 2004) for such ecosystems, also because their anthropomorphic features and behavioural traits lend themselves to provoking public sympathies (Polgar, 2008b; Kemp, 2005; Kutschera, 2006). Mudskippers could therefore be used to raise public awareness of the threats faced by mangals and to promote ecotourism to raise funds for ecosystem management and conservation (Ellison, 2000). This would assist the efforts to find a long term balance between human exploitation of mangroves' and mudflats' resources and their long term sustainability and ultimate survival.

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Species-area relationship and potential role as a biomonitor of mangrove communities of Malayan mudskippers

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Abstract The rapid and extensive destruction of mangrove forests and adjacent peritidal ecosystems in the Indo-Pacific region requires the development of efficient management and conservation actions. Mudskippers (Gobiidae: Oxudercinae) are amphibious gobies that are strictly linked to mangrove forests and tropical mudflats. I recorded the presence and habitat distribution of mudskipper species in four mangrove ecosystems along the west coast of Peninsular Malaysia. Different localities host different mudskipper communities, while in each locality species are differentially distributed along the intertidal gradient. At the ecosystem level, I found a significant exponential correlation between sampled area and the species richness of these communities, consistent with the SAR hypothesis. At the habitat level, the presence of a vertical zonation along the intertidal gradient suggests the possibility of using the species living exclusively in higher or lower levels as bioindicators for habitat anthropogenic impact, respectively from the land and from the sea.

Keywords Habitat destruction · Biodiversity loss · Mangals · Tropical intertidal zones · *Boleophthalmus* · *Periophthalmodon* · *Periophthalmus*

Introduction

As recently as 40–50 years ago, mangrove forests, or mangals (Macnae 1968), occupied about 60–75% of low-energy tropical shorelines around the world (Por and Dor 1984). Trend analyses show that the world has lost approx. 5 million ha of mangrove forests in the years 1980–2000, that is 20% of the total extent (Wilkie and Fortuna 2003). In South-East Asia, some of the most diverse mangrove formations in the world suffer from the highest loss rates of vegetation coverage (up to 2% per year: Wilkie and Fortuna 2003), mainly as a result of habitat destruction (Hogarth 1999; Menasveta 1996; Sasekumar 1980, 1991; Tomlinson 1986). The economic value of these ecosystems should be considered from a holistic perspective, since coastal reclamation and mangrove deforestation have far-reaching effects (Duke and Wolanski 2001). Rational management is being attempted; nevertheless, with the exception of ecotourism, the majority of these initiatives turned out to be largely unsustainable (Ellison 2000). At the same time, a firm political decision to reduce deforestation and the concrete instrumentation of management plans are largely lacking.

Oxudercine gobies (Teleostei, Gobiidae, Oxudercinae), also known as 'mudskippers', include abundant and typical resident species of mangrove

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and mudflat ecosystems throughout the Indo-Pacific region and along the Atlantic African coasts (Murdy 1989). These fishes present different degrees of adaptation to the amphibious lifestyle, and have colonised the entire intertidal gradient, from the subtidal to the supratidal zone (Nursall 1981; Murdy 1989; Takita et al. 1999; King and Udo 1997).

Data from a preliminary survey of the distribution of Malayan mudskipper species are here presented and discussed, to support the use of mudskippers as biomonitors of the health of mudflats and mangrove forests.

Materials and methods

A sampling survey was made in August 1996 in four localities distributed along coast in western Peninsular Malaysia: Tanjung Tuan (Lat 2°24′52″ N, Long 101°51′15″ E), Morib (Lat 02°45′33″ N, Long 101°26′16″ E), Sungai Sementa Besar (Lat 03°04′51″ N, Long 101°21′35″ E), and Kuala Selangor (Lat 03°19′32″ N, Long 101°14′20″ E). These localities are distributed within approximately 130 km (Fig. 1).

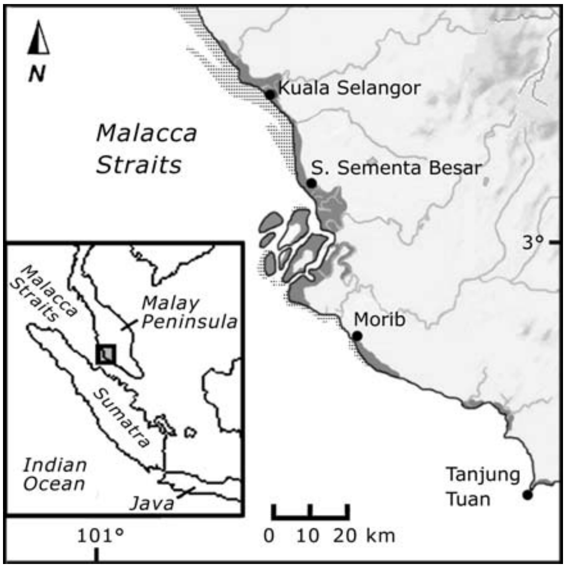

Fig. 1 Coastal areas of Western Peninsular Malaysia. *Shaded areas*: mangrove forests; *stippled areas*: tidal flats exposed at low tide (modified from Coleman et al. 1970); *large dots*: study localities

In each locality the horizontal extent of the intertidal zone was measured at spring low tide by use of GPS, tape meter and compass, moving from land to water. The distance was measured from the highest reaches of the mangrove forest (reclamation bund), to a point 10 m beyond the pneumatophore zone, at the edge of the low forest's marine fringe. In each locality I recorded the presence of mudskipper species in several plots along the intertidal gradient, within strip transects 25 m wide. The transects' total area was calculated by means of satellite images (Google Earth Plus, v. 4.2). I made a qualitative classification of mudskipper habitats based on plant zonation and on the presence of different water bodies and sedimentary deposits (Sasekumar 1980; Macnae 1968; Ricci Lucchi and Mutti 1980). In particular, 'estuarine tidal mudflats' are extended tidal mudflats associated with estuaries; and 'creek mud banks' are unvegetated mud banks of permanent tidal creeks. 'Low forests' are pioneer arboreal plant associations colonising the lower levels of the intertidal zone (*Avicennia* spp. and *Sonneratia* spp. zone: Sasekumar 1980); while 'high forests', are more terrestrial arboreal mangrove associations, found at higher levels (*Rhizophora* spp. and *Bruguiera* spp. zone: Sasekumar 1980). 'High inlet networks' are here defined as typical braided networks of ephemeral tidal waterways (inlets, gullies), which are flooded only by spring tides (Macnae 1968, pers. obs.). These networks are found in the higher portion of the high forest zone, where the empty beds are characterised by wetter and softer sediments at low tide. Typically in Malayan systems, this zone is also characterised by the mud mounds built by *Thalassina anomala* (Decapoda: Thalassinidae), and colonized by *Achrostichum* spp. halophytic ferns.

The presence of mudskipper species was determined during about 40 h of direct observation by naked eye or binoculars (Pentax 8 × 40), at distances of 2–10 m, at low tide (LT ± 2 h), and between 9 am and 7 pm hours. All sites were surveyed during spring tides; in Kuala Selangor, forested areas were also visited during neap tides. A reference collection was made by using hand nets, bait and line, stake nets and cast nets. Collected specimens were morphologically examined in the laboratory to verify the efficiency of visual discrimination in the field (Murdy 1989; Murdy and Takita 1999; Takita et al. 1999; Darumas and Tantichodok 2002). A sample was fixed in 10% formalin, preserved in 70% ethanol, and deposited to the Genoa Natural History Civic

Museum (MSNG). More material was subsequently collected in the same areas by Dr. M.Z. Khaironizam in Sementa and in Kuala Selangor (Khaironizam and Norma-Rashid 2002; Polgar and Khaironizam 2008, in press).

To minimize the effects of ecological partitions between different age classes (Nursall 1981; Clayton and Vaughan 1988; Clayton 1993), only the presence of larger individuals was recorded (SL $\geq$ 50% of the maximum recorded standard length: Murdy 1989; Murdy and Takita 1999; Takita et al. 1999; Darumas and Tantichodok 2002; Swennen and Ruttanadakul 1995; Khaironizam and Norma-Rashid 2002). This also increased the reliability of discrimination, since diagnostic morphological characters and colouration traits of smaller individuals are often not discernible in the field.

In order to record the habitat distribution of mudskipper species, each strip transect was divided into two zones along the intertidal gradient: (1) high forest and (when present) high inlets' network (HF); and (2) low forest and (when present) unvegetated mudflat (LF). At low tide, mudskippers are differentially distributed, and three species assemblies are observed: (1) species found only in low forests and/or on mudflats and creek mud banks (SLF: found in the LF zone); (2) species found only in high forests (SHF: found in the HF zone); and (3) species found in both zones (SIF) (Fig. 2).

Results and discussion

The surveyed ecosystems presented wider intertidal zones and higher habitat diversities moving from south to north, from Tanjung Tuan to Kuala Selangor. Therefore, strip transects had proportionally larger areas (Fig. 1, Table 1).

Nine species of mudskippers and their habitat distribution were recorded (Table 2): *Boleophthalmus boddarti* (Pallas, 1770); *Boleophthalmus* sp.; *Periophthalmodon schlosseri* (Pallas, 1770); *Periophthalmus argentilineatus* Valenciennes, 1837; *P. chrysospilos* Bleeker, 1852; *P. gracilis* Eggert, 1935; *P. novemradiatus* (Hamilton, 1822); *P. spilotus* Murdy and Takita 1999; and *P. walailakae* Darumas and Tantichodok

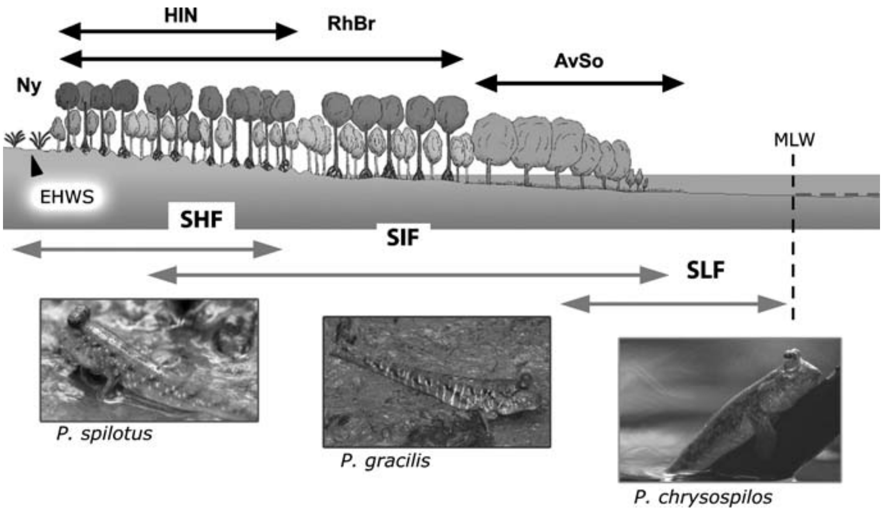

Fig. 2 Distribution of oxudercine species along the intertidal zone. Mangrove ecosystems and mudflats are the natural habitat of amphibious oxudercine gobies, or 'mudskippers' (Gobiidae: Oxudercinae). Adults have species-specific habitat distributions along the intertidal gradient at low tide. There are species which are found only in high forests and high inlet networks (*SHF*); species found in low forests and/or on unvegetated mud areas (*SLF*); and species found in both zones (*SIF*). As an example, three species of the genus *Periophthalmus* (=*P.*) are illustrated (photos by G. Polgar: *P. spilotus*: Malaysia, Kuala Selangor, 1996; *Periophthalmus gracilis* and *Periophthalmus chrysospilos*: Malaysia, Pulau Kukup, 2007). EHWS = Extreme High Water Springs; MLW = Mean Low Waters. Mangrove zonation modified from Macnae (1968): AvSo = *Avicennia* and *Sonneratia* zone; RhBr = *Rhizophora* and *Bruguiera* zone; HIN = high inlet network; mounds of *Thalassina anomala* are illustrated in this area; Ny = belt of *Nypa fruticans* at the landward edge (this zone is usually lacking due to land reclamation)

Table 1 Extent of the intertidal zone, area of strip transects and habitat diversity in the surveyed localities

Locality	Ext. (m)[a]	Area of strip transects (m^2)	Number of plots	Estuarine tidal mudflats	Creek mud banks	Low forests	High forests	High inlet networks
Tanjung Tuan	30	750	2			X	(X)[b]	
Morib	120	3,000	3		X	X	(X)	
Sungai Sementa Besar	200	5,000	2		X	X	X	X
Kuala Selangor	450	11,250	4	X		X	X	X

Systems with wider intertidal zones have higher habitat diversities

[a] Average linear horizontal extent of the intertidal zone

[b] In parentheses, relatively small stands of *Rhizophora* spp., behind the pioneer zone. See text for other definitions. “X” indicates presence

Table 2 Presence-absence matrix and habitat distribution of the mudskipper species in the surveyed plots

Plots[a]	Zone[b]	Arg[c]	Bod	Chr	Gra	Nov	Sch	Spi	Wal	*Boleophthalmus* sp.
T1	LF	**1**	0	0	**1**	**1**	0	0	0	0
T2	(HF)[d]	0	0	0	**1**	0	0	0	0	0
M1	LF	0	**1**	**1**	0	0	**1**	0	0	0
M2	LF	**1**	0	0	**1**	0	0	0	**1**	0
M3	(HF)	0	0	0	**1**	0	0	0	**1**	0
S1	LF	0	**1**	**1**	0	0	**1**	0	0	0
S2	HF	0	0	0	**1**	**1**	0	**1**	**1**	0
K1	LF	0	0	0	0	0	0	0	0	**1**
K2	LF	0	**1**	**1**	0	0	**1**	0	0	0
K3	HF	0	0	0	**1**	**1**	0	**1**	0	0
K4	HF	0	0	0	0	**1**	**1**	0	**1**	0

[a] T: Tanjung Tuan; M: Morib; S: Sungai Sementa Besar; K: Kuala Selangor

[b] LF: low forests, mudflats and creek mud banks; HF: high forests and high inlet networks

[c] Mudskipper species are indicated by the first three letters of their specific scientific name; *B.* sp. indicates the unidentified species of *Boleophthalmus*; 1 = presence; 0 = absence

[d] In parentheses, relatively small stands of *Rhizophora* spp., behind the pioneer zone

2002. All the observed and collected species are consistent with the available keys (Murdy 1989; Larson and Takita 2004) and with field identification notes by Takita et al. (1999). In particular, the behavioural and colouration traits of *B.* sp. (found only in Kuala Selangor), are consistent with the species identified by Takita et al. (1999) as *Boleophthalmus dussumieri* Valenciennes, 1837 from the same locality. Nonetheless, subsequent surveys and collections made in Johor, Malaysia and examination of additional material obtained from Kuwait, Iran, Taiwan and Southern China, suggest that this Malayan species is not *B. dussumieri*, but *Boleophthalmus pectinirostris* (Linnaeus, 1758). No specimen of this species was collected in Kuala Selangor, and further taxonomic studies are being made to confirm this record.

Four species were exclusively found in more aquatic habitats (SLF); one species was only found in more terrestrial ones (SHF); and four species were found in both habitats (SIF: Table 3). Among SIF species, *P. novemradiatus* was found in LF habitats only once (Tanjung Tuan), while *Pn. schlosseri* was found in HF habitats only in one locality (Kuala Selangor) (Table 2).

The number of species (n) is positively correlated to the total surveyed area (A) in each locality (Tables 1, 3). In particular, the scatter plot of n against A fits an exponential curve (on a log-log plot,

Table 3 Mudskipper species assemblies in the surveyed localities

Species	Species assembly	Tanjung Tuan	Morib	Sungai Sementa Besar	Kuala Selangor
P. spilotus	SHF[a]			X	X
P. novemradiatus	SIF[b]	X		X	X
P. gracilis	SIF	X	X	X	X
P. walailakae	SIF		X	X	X
Pn. schlosseri	SIF		X	X	X
P. argentilineatus	SLF[c]	X	X		
P. chrysospilos	SLF		X	X	X
B. boddarti	SLF		X	X	X
Boleophthalmus sp.	SLF				X
Totals[d]		3 (0, 2, 1)	6 (0, 3, 3)	7 (1, 4, 2)	8 (1, 4, 3)

[a] Species found only in high forests and high inlet networks

[b] Species found in both zones

[c] Species found only in low forests and on unvegetated mud

[d] Total number of species found in each locality (in parentheses, number of species in each assembly: high forest, both zones, low forest)

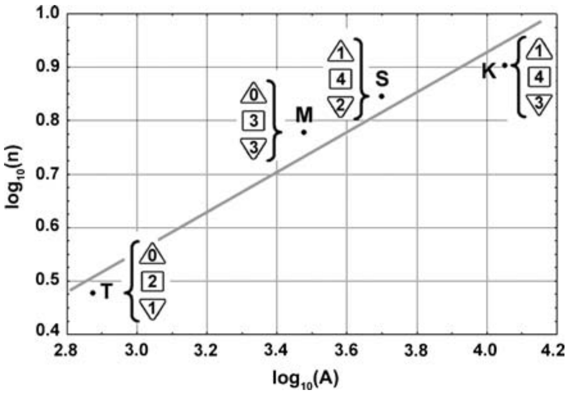

Fig. 3 The number of species (n) plotted against the explored areas at low tide (**a**), on a log-log plot (slope = 0.37; $r = 0.97$; P (uncorr) < 0.05). T = Tanjung Tuan; M = Morib; S = Sungai Sementa Besar; K = Kuala Selangor. Numbers in triangles pointing upward = number of species found only in high forests and high inlet networks; numbers in triangles pointing downward = number of species found only in low forests and on unvegetated mudflats; numbers in squares = number of species found in both zones

$r = 0.97$; $P < 0.05$), with a power factor (z) of 0.37 (Fig. 3; STATISTICA v. 6.0 © StatSoft, Inc.), consistent with the SAR hypothesis (Species-Area-Relationship: Rosenzweig 1995).

The SAR is a classical ecological concept and a general descriptor at ecosystem level. In this case, mangrove forested areas are considered as “islands” separated by distance along coast. SAR’s ecological interpretation is often controversial (Rosenzweig 1995; Gotelli and Ellison 2004). In very general terms, z exponents of 0.2–0.4 can derive from differently clustered distributions of conspecific individuals, which is in accordance with the observed vertical zonation, and from abundance distributions similar to lognormal ones with extra rarity, widespread among very different organisms (Martín and Goldenfeld 2006).

Under constant tidal conditions and within a given mangrove ecosystem, the composition of the mudskipper community (i.e. the number of species) drastically changes along the intertidal gradient, while compositional changes in a perpendicular direction seem negligible. If future studies will confirm and generalise this pattern, and if a SAR exists between different systems, the species numbers will be in an exponential relationship not only with areas, but also with the *linear* extent of the respective intertidal zones.

At the ecosystem level, the decreasing number of species from north to south seems to be related to the distribution of available habitats along coast. In the Straits of Malacca the formation of mudflats is determined by the high sediment loads discharged by fluvial systems (e.g. Klang-Langat and Selangor rivers) and by the pattern of currents along coast, which are predominantly directed from SE to NW (Coleman et al. 1970, Fig. 1). The distribution of mudskipper species suggests both an interaction between habitat availability and larval dispersal processes, influenced by the pattern of currents, and the existence of specific mechanisms of habitat selection (Begon et al. 1986). On the other hand, these results also imply that an anthropogenic reduction of available habitats by deforestation and land reclamation would result in a decrease of the number of mudskipper species.

At the habitat level, the zonation of mudskipper species suggests a species-specific selection of environmental conditions along the intertidal gradient, even if the role of synecological factors, such as direct and indirect intra- and interspecific competition, is almost completely unknown (Nursall 1974, 1981; Milward 1974). As a general rule, only a minority of aquatic intertidal species is able to cope with the harsh conditions found in the supratidal zone (Raffaelli and Hawkins 1999). Supratidal mangrove zones host fewer and evolutionarily peculiar species, such as *P. spilotus* and *P. novemradiatus*. Since higher zones are also more impacted by anthropogenic habitat destruction, such species can be used as key-indicators of the effects of habitat destruction of mangrove ecosystems from land. Unfortunately, no such study is currently available. In peninsular Malaysia, the freshwater species *Periophthalmodon septemradiatus* (Hamilton, 1822), presently known only from a village nearby Kuala Selangor (Khaironizam and Norma-Rashid 2003), is maybe the most terrestrial and one of the most endangered Malayan mudskipper species. Instead, species living exclusively at lower levels can be key-indicators of disturbing factors acting primarily from the sea, such as pollution (e.g. Nakata et al. 2002; Kruitwagen et al. 2006).

Narrower intertidal zones have also lower habitat diversity (Table 1). The decreased species richness may thus be a consequence of reduced habitat availability. Nevertheless, not only do species living exclusively at higher or lower levels (SHF and SLF) generally decrease in number in systems with narrower intertidal zones, but so do ubiquitous species (SIF). Almost all species assemblies contain the same or a higher amount of species in localities with wider intertidal zones (Table 3, Fig. 3).

This suggests that some factor correlated to the total available area is acting at ecosystem level, affecting whole mudskipper communities.

Further investigations may quantify whether the observed SAR is mainly caused either by a simplification of ecosystem structure and a reduction of habitat availability in areas with narrower intertidal zones, or by the influence of coastal currents and physiography on larval dispersal patterns.

Species' reference collection

Boleophthalmus boddarti (Pallas, 1770): **1** specimen from 1 locality, Selangor, MALAYSIA: Sementa, Sungai Sementa Besar; MSNG 54124 (108 mm SL), exposed creek's mud banks, 30 August, 1996. *Periophthalmodon schlosseri* (Pallas, 1770): **1** specimen from 1 locality, Selangor, MALAYSIA: Kuala Selangor; MSNG 54125 (159 mm SL), mangal high forest (*Bruguiera* and *Rhizophora* spp. zone), 09 August, 1996. *P. argentilineatus* Valenciennes, 1837: **1** specimen from 1 locality, Negeri Sembilan, MALAYSIA: Tanjung Tuan; MSNG 54126 (46 mm SL), *Sonneratia* spp. pioneer shore, 15 August, 1996. *P. chrysospilos* Bleeker, 1852: **3** specimens from two localities, Selangor, MALAYSIA: Morib and Pulau Kelang; size range 65.2–74.3 mm SL: MSNG 54128, **2** (65, 74 mm SL), *Sonneratia* spp. pioneer shore, Morib, *ibid.*, 17 August, 1996; MSNG 52024, **1** (74 mm SL), lower mud flat, northern coast of Pulau Kelang, *ibid.*, 30 August, 1996. *P. gracilis* Eggert, 1935: **6** specimens from 4 localities, MALAYSIA, Selangor and Negeri Sembilan: Kuala Selangor, Pulau Kelang, Morib, and Tanjung Tuan; size range 27–40 mm SL: MSNG 54129, **2** (27, 30 mm SL), mixed mangrove forest, high inlet network, Kuala Selangor, *ibid.*, 27 August, 1996; MSNG 54130, **1** (27 mm SL), mixed mangrove forest, Pulau Kelang, *ibid.*, 30 August, 1996; MSNG 54131, **1** (33 mm SL), *Rhizophora* spp. forest, Morib, *ibid.*, 17 August, 1996; MSNG 54132, **2** (31, 40 mm SL), *Sonneratia* spp. pioneer shore and *Rhizophora* spp. forest, Tanjung Tuan, *ibid.*, 15 August, 1996. *Periophthalmus novemradiatus* (Hamilton, 1822): **6** specimens from 3 localities, MALAYSIA, Selangor and Negeri Sembilan: Kuala Selangor, Pulau Kelang, and Tanjung Tuan; size range 40–57 mm SL: MSNG 54133, **1** (44 mm SL), *Bruguiera* spp. high forest, nearby the reclamation bund, Kuala Selangor, *ibid.*, 08 August, 1996; MSNG 54134, **2** (40, 57 mm SL), mangrove mixed forest, high inlet network, Kuala Selangor, *ibid.*, 27 August, 1996; MSNG 54135, **2** (40, 49 mm SL), inside and in front of a *Rhizophora* spp. stand, Pulau Kelang, *ibid.*, 30 August, 1996;

MSNG 54136, **1** (52 mm SL), *Sonneratia* spp. pioneer shore, Tanjung Tuan, *ibid.*, 15 August, 1996. *Periophthalmus walailakae* Darumas & Tantichodok, 2002: **1** specimen from 1 locality, Selangor, MALAYSIA: Kuala Selangor; MSNG 51393, (109 mm SL), *Bruguiera* spp. high forest, 8 August, 1996.

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Title:

Multivariate characterization of the habitats of seven species of Malayan mudskippers (Gobiidae: Oxudercinae).

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Abstract

Mudskippers (Gobiidae: Oxudercinae) are highly amphibious and closely related tropical gobies. We recorded the occurrence and habitats of seven sympatric species of Malayan mudskippers: *Boleophthalmus boddarti*, *Boleophthalmus pectinirostris*, *Periophthalmodon schlosseri*, *Periophthalmus chrysospilos*, *Periophthalmus gracilis*, *Periophthalmus novemradiatus* and *Scartelaos histophorus*. 14 surveys were made in six localities along 120 km of coasts of western peninsular Malaysia in August 1996 and September 2006. A multivariate set of ordinal parameters was used to quantify different levels of water availability in mudskippers' habitats, and three guilds of species could be discriminated. The resulted pattern mirrored that one depicted by physiological and anatomical studies, although it did not perfectly match the systematic pattern. Differentiation of trophic niches may also play a role in the habitat selection of the herbivorous *Boleophthalmus* species, which were only found in unvegetated and illuminated areas, and on fine sediments. Within the more terrestrial guild, a significantly negative co-occurrence suggest the presence of direct interspecific competition.

Keywords: adaptation; terrestriality; habitat differentiation; water to land transition; mudskippers

Introduction

Except for tetrapods, semi-terrestrial habitats offer challenging conditions to living vertebrates, and only a small number of these species presents truly amphibious behaviours.

Mudskippers (Teleostei: Gobiidae: Oxudercinae: Periophthalmini) are gobies which are "fully terrestrial for some portion of the daily cycle" (Murdy, 1989). These species are included in four genera: *Boleophthalmus* Valenciénnes, 1837; *Periophthalmodon* Bleeker, 1874; *Periophthalmus* Bloch and Schneider, 1801; and *Scartelaos* Swainson, 1839. All of the 29 mudskipper species (Larson and Takita, 2004) are resident intertidal fishes (Gibson, 1982), and are strictly linked to temperate, subtropical and tropical mudflat, mangrove and lower fluvial ecosystems. They are distributed along the Atlantic coasts of Africa and in the entire Indo-West-Pacific region (Murdy, 1989).

Although based on few model species, anatomical and physiological studies show a gradual increase of the degree of adaptation to semi-terrestrial conditions, from *Scartelaos* to *Boleophthalmus*, through *Periophthalmodon* and *Periophthalmus* species (e.g. Milward, 1974; Low et al., 1990; Kok et al., 1998; Ip et al., 1990; 2001; Zhang et al., 2000; 2003; Aguilar, 2000; Chew et al., 2003). When this pattern is compared with the current phylogenetic hypothesis (Murdy, 1989), species of morphologically derived genera present higher degrees of terrestrial adaptation, suggesting an increasing adaptation to terrestriality at each cladogenetic event (**EMS**, **S1**). Sympatric mudskipper species of different genera are generally found in different habitats along the intertidal zone (Milward, 1974; Nursall, 1981; Swennen et al., 1995; Takita et al., 1999). Habitat descriptions of mudskipper species are generally consistent with this scenario, with species of more derived genera occurring in habitats with lower levels of water. Nonetheless, quantitative studies are lacking.

During this study, seven species of mudskippers included in four genera with different degrees of terrestrial adaptation were recorded in the intertidal ecosystems of six different locations along the west coast of the Malay Peninsula. The oxudercine species recorded from this region are listed in **Tab. 1**. The main objectives of this study are: 1) explore the correspondence between mudskipper species and a multivariate measure of water availability in their habitats; and 2) test for non-random patterns in species' co-occurrence among locations and sites, to search for possible synecological interactions.

Materials and methods

Fieldwork and species

Presence of mudskippers was recorded during about 44 h of direct observations by binoculars or naked eyes, at distances of 2-10 m, and between 9 am and 7 pm hours. 14 surveys (02 Aug-30 Aug 1996; and 28 Sep 2006) were made along the intertidal zone, on foot (forested areas) or by boat (mudflats and creeks). A reference collection was made to check for the efficiency of visual discrimination to species level in the field (Murdy, 1989; Murdy and Takita, 1999; Takita et al., 1999; Darumas and Tantichodok, 2002). Inside mangrove forests, fish were captured by hand net or bait and line, while stake nets and cast nets (Gibson, 1999) were used along forest marine fringes, on mud banks and tidal flats. A sample was fixed in 5% formalin, preserved in 70% ethanol, and deposited to the Genoa Natural History Civic Museum (MSNG), and several museum specimens were examined (**ESM, S2**).

Seven species were studied: *Boleophthalmus boddarti* (**Fig. 1**, **l**); *Boleophthalmus pectinirostris* (**Fig. 1**, **m**); *Periophthalmodon schlosseri* (**Fig. 1**, **k**); *Periophthalmus chrysospilos* (**Fig. 1**, **j**); *Periophthalmus gracilis* (**Fig. 1**, **h**); *Periophthalmus novemradiatus* (**Fig. 1**, **i**); and *Scartelaos histophorus* (**Fig. 1**, **n**). *S. histophorus* and site M5 were added to the dataset after a survey in September 2006, and several specimens were collected in the same site in May 2007; *B. pectinirostris* was observed in site S8 (K.Selangor) in August 1996, and several specimens were collected in Tg. Piai, Johor, in September 2006 (**ESM**, **S2**). Isolated records of *P. walailakae* and *P. argentilineatus* in 1996 were not included in this analysis (**EMS**, **S2**; Polgar & Khaironizam, in press). Other specimens of these two latter species, of *P. spilotus* (= *Periophthalmus* sp. in Takita et al., 1999, **Tab. 1**), *Pn. septemradiatus*, and *Pd. elongatus* were subsequently collected by the first author and Dr. M.Z. Khaironizam in the same or neighbouring areas (**EMS**, **S2**; Khaironizam and Norma-Rashid 2002; 2003; Polgar, 2008).

Six locations and 26 sites along the whole intertidal zone, including mangrove forests and adjacent peritidal ecosystems were visited during August 1996 and September 2006 (**Fig. 2**). Namely, Kuala Selangor (03°20.5' N, 101°14.5' E); Sungai (= river) Sementa Besar (03°5.0' N, 101°21.5' E); Pulau (= island) Kelang (03°3.5' N, 101°19.0' E); Jugra (02°49.0' N, 101°25.0' E); Morib (02°45.5' N, 101°26,3' E); and Tanjung (= cape) Tuan (= Cape Rachado: 2°24.9' N, 101°51.3' E). These locations are distributed along 120 km of the west coast of the Malay Peninsula. The presence of mudskipper species was recorded in each site, within small areas of few square meters, in homogeneous environmental conditions.

All sites were surveyed ± 3 days around spring tides; K. Selangor forests were also surveyed ± 3 days around neap tides. We used tidal data from three reference stations (Hydrographic Directorate, RMN 1996): Port Klang (for Kuala Selangor, P. Kelang and Sg. Sementa Besar); Permatang Sedepa (for Jugra and Morib); and Port Dickson (for Tanjung Tuan). The Malacca Straits is a semidiurnal meso-macrotidal system, and tidal ranges of approx. 1-2 m during neaps and 4-5 m during springs. The formation of tidal mudflats, which within the explored tract of coast decrease in extension from north to south, is determined by the high sediment loads discharged by fluvial systems (e.g. Klang-Langat and Selangor rivers) and by the pattern of currents along coast, which are predominantly directed from SE to NW (Coleman et al., 1970). Salinity in the Straits varies with rainfall, with average values of 28-32 ppt, and two minima in May and November (southern and northern monsoons, respectively: Sasekumar, 1980). Within the intertidal zone (creeks, mudflats, mangroves) salinity fluctuations are determined by rainfall, evapouration and tidal action, becoming more extreme towards land (approx. 3-50 ppt: Sasekumar, 1974; 1994).

In laboratory, fish were measured by a dial caliper to the nearest mm; in the field, fish approx. size was visually estimated by using static objects of known size as a scale (e.g. leafs, sticks). To minimize the effects of ecological partitions between different size classes

(Clayton and Vaughan, 1988; Clayton, 1993), we analysed only records of larger individuals [hereafter indicated as ‘adults’: SL approx. ≥ 50% of the maximum recorded standard length (SL_{max})]. This also increased the reliability of identification.

Spatial and temporal distribution

To compare the terrestriality of different species, we recorded mudskippers and their habitats during both low and high tide. In the first case, observations were made ±2 hours around the predicted low tide (*phase X*), and when waterways and basins inside the study area were not influenced by the weather. During this phase, the species’ distribution was assumed to be unaffected by the movement of water. In the second case, we observed mudskippers when reached by the water during flood tide: during this phase mudskippers perform diverse behaviours to avoid the water column, either out of water, or inside burrows (Harris, 1960; Sayer, 2005; Colombini et al., 1995; Ishimatsu et al., 1998; Lee et al., 2005).

Habitat water availability and species occurrence

We quantified the environmental water present in mudskippers’ habitats using five ordinal parameters: density of vegetation coverage (VC); water bodies (WB); tidal influence (TI); structural elements (SE); and external medium (EM). The former four parameters were measured during low tide, while the last one was measured when mudskippers were reached by the flood tide. All parameters have higher values in more terrestrial conditions (**Tab. 2**, **Fig. 1**, **a**-**g**).

To discriminate between different densities of vegetation coverage (VC), we followed a modified version of the Malayan mangrove intertidal zonation by Sasekumar (1980). Vegetation coverage limits air movement and light penetration, and reduces the evaporation rate at ground level (Macintosh, 1977; Clayton and Snowden, 2000). Therefore, increasing

VCs (Sarpedonti and Sasekumar, 1996) reduce evaporation rates on the exposed substrate. We observed six different levels of VC.

Water bodies (WB) are different hydrogeomorphic and bioturbation structures which contain water. Their morphology and persistency depends on weather, tidal influence, wave action, and biological activity. On soft bottomed accreting shores, during low tide they present a typical distribution along the intertidal zone (**Fig. 3**). Wider and deeper WB within a given site both determine more aquatic conditions, and imply lower energetic costs to find water for mudskippers. We observed four different WB.

Field observations of the water's edge were compared to tide tables, and mean elevations (tidal datums) calculated. Then we recorded the datum reached by the water when each site was partially or completely submerged (TI). This parameter is associated with the frequency of submersion and the minimum time interval between wettings (Sasekumar, 1974). Lower levels imply higher frequencies, shorter intervals, and more aquatic conditions. Four TI levels characterised the study sites.

Biotic and abiotic objects or structural elements (SE) whose size is comparable to mudskippers (4-25 cm total length), can greatly increase substrate heterogeneity. More heterogeneous substrates have a greater surface per square meter, thus increasing humidity at ground level, and the amount of capillary water. The presence and composition of SE along soft-bottomed tropical intertidal zones depends on vegetal debris and mangrove diversity (pencil-like pneumatophores, propagules, prop roots, knee-roots, etc.; Tomlinson, 1986). We observed a general increase of the diversity and size range of SE from sea to land, and recorded four different combinations of SE.

External medium (EM) during flood and high tide was determined by the different strategies of water column avoidance. During flood tide, fishes were observed either to enter burrows (aquatic conditions: EM= 1), or to come out of water (semi-terrestrial conditions:

EM= 2; **Tab. 2**). This behaviour was species-specific (**Tab. 3**): during high tide, EM1 species were never observed out of water, thus we assumed that these species remained below water. Even if it was not observed, it is possible that some specimens of EM2 species entered burrows during flood tide and remained below water during high tide (e.g. male egg-guarders like *P. chrysospilos*: Macnae, 1968).

To relate species occurrence (modalities: columns) and environmental parameters (observations: rows), we performed a multiple correspondence analysis (MCA: XLSTAT 7.5.3 © Addinsoft). MCA, also known as homogeneity analysis, is an exploratory technique alternative to PCA for categorical or ordinal variables (Jobson, 1992). The matrix (**ESM**, **S5**) was built from the matrices of species presence/absence and environmental parameters (**Tab. 3**), by recording the presence/absence of each species in the conditions described by each value of the parameters. To corroborate the species associations in the factorial space, we analysed the same matrix by agglomerative hierarchical clustering (AHC: XLSTAT 7.5.3 © Addinsoft), adopting the Jaccard similarity index and the strong linkage aggregation method (Johnson and Wichern, 1992). Finally, jackknife protocol was applied, to search for influential observations (parameters' values) that could bias the analysis (Ellison and Gotelli, 2004).

Co-occurrence

We used the 'Co-occurrence' module of ECOSIM (ECOSIM 7.72 © Kesey-Bear and Acquired Intelligence, Inc.: Gotelli and Entsminger, 2001) to test for non-random patterns of species co-occurrence among different locations or sites. In the null-model simulation (5,000 reps), the total number of occurrences of each species and the number of species in each site or location were set to be the same as in the original data set. The C-score index (Stone and

Roberts, 1990) was used. These settings were shown to be among the least vulnerable to Type I errors (Gotelli and Entsminger, 2001).

Results

Mudskipper species

All the observed and collected species are consistent with the most recent taxonomic keys and field identification notes (Murdy, 1989; Takita et al.,1999; Larson and Takita, 2004). In particular, the identification of *B. pectinirostris* was based on observations in the field and supported by subsequent surveys and collections made by the first author in Johor, Malaysia, and examination of additional material of both species obtained from Kuwait, Iran, Bangladesh, India, Malaysia, Taiwan, China and Japan (**EMS**, **S2**).

Predorsal scale counts and longitudinal scale counts can discriminate *B. pectinirostris* from *B. boddarti* and *B. dussumieri* (Murdy, 1989), while we could not use Murdy's key, due to the largely overlapped ranges of anal and D2 fin element's counts in these species.

In the field, Malayan *B. pectinirostris* were identified thanks to the lower portion of the eyeballs, which is pale blue and well visible in larger specimens; and the presence of black blotches along flanks which never extended below the lateral midline. *B. boddarti* presented several black diagonal bars extending well below the lateral midline, and neutrally coloured eyeballs. Moreover, both *B. boddarti* and *B. pectinirostris* had flecks of colour behind D1 spines, contrasting with the background of the interradial membrane: these flecks are paler than the background in *B. boddarti*, while they are darker than the background in *B. pectinirostris*. In live or freshly dead specimens, the D1 basal membrane of Malayan young *B. boddarti* is yellow, while that one of *B. pectinirostris* is dark grey. The black dorsal margin of the pectoral fins, absent in *B. pectinirostris*, is usually difficult to spot in the field, but diagnostic (Takita et al., 1999; pers. obs.).

In all the collected specimens, adult females of both species had the 2nd and 3rd D1 spines which were more elongated than in males.

Field observations, and correspondence between species and habitat water availability

The first two factorial axes of the MCA accounted for 73.7% of total variance (**Fig. 4a**). All the species (modalities) had significant test values (alpha= 0.05; two-tailed test) in the F1, F2 factorial space. Three groups of species were supported both by the MCA and the AHC (cut-off similarity value= 0.6: **Fig. 4b**).

Group A included *P. novemradiatus* and *P. gracilis* (node's similarity= 0.85); this group was associated with denser vegetation coverage (VC3-5); higher spatial heterogeneity (SE3,4); areas flooded at least by the mean high water spring (TI3); bottoms of ephemeral waterways (WB3); and with subaerial conditions during the high tide (EM2). *P. gracilis* was found in wet, relatively open pioneer mangrove forests; on higher levels, it was only found on the bottom of ephemeral inlets, often densely aggregated around the large burrow openings of *Pn. schlosseri*, filled with water. By contrast, *P. novemradiatus* was found everywhere inside the forest, up to the supratidal zone (VC6), where it was the only mudskipper species present during low tide conditions (**Tab. 3**, **EMS**, **S3**, **S4**). Only one individual was observed using a turreted burrow, in the *Rhizophora* and *Bruguiera* zone.

Group B included only *Pn. schlosseri*, which was weakly related to group A (node's similarity= 0.52); this species was associated with tide pools (WB2); areas flooded at least by the mean high water neap (TI2); and subaerial conditions during the high tide (EM2). It was found on the banks of creeks and run-off channels; on open mudflats; and inside mangrove forests (**Tab. 3**, **EMS**, **S3**, **S4**). In this last case, adults were always found nearby the typical large openings of their burrows, typically disposed along the bottom of ephemeral inlets; here we also found very young individuals (< 4 cm TL).

Group C included *S. histophorus*, *B. boddarti*, *B. pectinirostris*, and *P. chrysospilos* (node's similarity= 0.57); this group was associated with the absence of vegetation coverage (VC1); absence of structural elements (SE1); and the presence of larger water bodies (WB1). During low tide, adults of *S. histophorus*, *B. boddarti*, and *B. pectinirostris* were always found in the lower intertidal zone, nearby the water edge (tidal creeks, tide pools, seaward edge: **Tab. 3**, **EMS**, **S3**, **S4**). Within C, *P. chrysospilos* was the only species which remained out of water during the high tide (EM2): in the AHC, it was the sister group of all the other species (**Fig. 4b**). During high tide, *P. chrysospilos* perched on pneumatophores, trunks and prop roots of the forest marine fringe; when the tide ebbed, it gathered in small shoals of 20-30 individuals which oriented themselves towards the sea and migrated towards the sea, eventually dispersing on the middle and lower mudflat. During flood tide, these fishes followed the water edge without forming shoals, and entered the pioneer forest marine fringe, climbing on emerged roots and trunks. Only few individuals were observed inside burrows, which in this species were excavated and maintained in open areas, just in front of the pioneer mangrove marine fringe.

Jackknifing changed only the topology of *P. chrysospilos* and *Pn. schlosseri*. These species always remained in intermediate positions, except when the EM1 or EM2 states were eliminated; in these cases, *P. chrysospilos* was more deeply included in group C.

Co-occurrence and possible synecological effects

Species composition significantly differed only among sites. Observed C-scores between locations were lower than expected by chance (positive co-occurrence), but not significantly ($p > 0.5$). Instead, observed C-scores between sites were significantly higher than expected by chance (negative co-occurrence: $p = 0.0004$). This would suggest the presence of competitive interactions between species among sites, i.e. along the intertidal zone.

Nonetheless, the results of the multivariate analysis suggest that this pattern could also reflect a 'habitat checkerboard' (Gotelli and Entsminger, 2001) with respect to water availability; that is, different species may occur together less frequently than expected by chance, simply because they live in very different habitats. To test this hypothesis, we analysed two separate matrices containing either the species of groups A+B, or the species of groups C+B (**Fig. 4a**). The observed C-scores were significantly higher than expected by chance only in the first case (negative co-occurrence: p= 0.002 vs. p= 0.098, respectively).

Discussion and conclusions

All the species recorded during this study were previously reported from the Malay peninsula. In particular, *B. pectinirostris* was recorded by Cantor (1849) and Koumans (1953) (**Tab. 1**). Murdy (1989) reported a lot (USNM 139356) of this latter species from Sabah (Insular Malaysia), but considered it as "questionable" on the basis of the distributional range of the other lots; he also reported another lot from the "East Indies" (USNM 12567), a name used for the Malay Archipelago. Takita et al. (1999), Khaironizam and Norma-Rashid-Y. (2005), and Polgar & Khaironizam (in press) reported *B. dussumieri* in the Malacca Straits due to Murdy's considerations. The larger *Boleophthalmus* sp. observed, but not collected by Murdy (1986) in Muar, Johor, Malaysia, may have been the same species observed by Takita et al. (1999). Our discrimination is based on the examination of several Malayan and non Malayan specimens of *B. boddarti*, *B. pectinirostris* and *B. dussumieri* (**EMS**, **S2**). Further taxonomic investigations are presently being made; if these results will be confirmed, the distributional range of *B. pectinirostris* would extend from southern Japan to South East Asia, while *B. dussumieri* would be limited to the Persian Gulf, Pakistan and the west coasts of India. *P. kalolo*, *P. malaccensis*, and *Pd. borneensis*, previously recorded along the coasts of the Malay peninsula by Murdy (1989) and Koumans (1953), were not recorded during this study (**Tab. 1**).

Indirect measures of habitat water availability mirrored mudskippers' physiological adaptations to terrestriality. *Boleophthalmus* and *Scartelaos* species are relatively less terrestrial, and were found in more aquatic conditions (group C: **Fig. 4a**). Takita et al. (1999) and Khaironizam and Norma-Rashid (2005) found *B. boddarti* also in intertidal areas covered by mangrove vegetation, while Swennen et al. (1995), reported this species among "scattered young mangrove trees". During this study (**ESM**, **S3**, **S4**), adults were observed only on unvegetated substrates: on the banks of an artificial lake (S1), in front of pioneer forest marine fringes (S7), on open mudflats (K4), and on exposed creek/river banks, a few meters from the vegetation (S9, S10, K1, B1, J1, M4). Nonetheless, during low tide, smaller specimens were frequently found well inside mangrove forests (VC4), on the bottom of ephemeral tidal inlets. In all other respects, the recorded habitats of *B. boddarti* and/or *S. histophorus* are consistent with the reports of Macnae (1968: *B. boddaerti*= *B. boddarti*; *S. viridis*= *S. histophorus*), Berry (1972), Sasekumar (1974), Frith et al. (1976), Nateewathana and Tantichodok (1984: *P. viridis*= *S. histophorus*), Murdy (1986: *S. pectinirostris*= *S. histophorus*), and Takita et al. (1999), along the west coasts of the Malay peninsula; with the study of Swennen et al. (1995), on the east coasts of the Malay peninsula; and with the accounts of Milward (1974), Townsend and Tibbetts (1995; 2005), and Aguilar (2000) on the Australian populations of *S. histophorus*. The observed habitat of *B. pectinirostris* is consistent with the observations made by Takita et al. (1999) in the Malacca Straits, and by Yang (1996) and Hong et al. (2007) in China. It is worth noting that the well-supported occurrence of *Boleophthalmus* spp. in habitats with relatively high levels of water availability, at lower intertidal levels and with no vegetation coverage, may also reflect their trophic specialization. Adults of *Boleophthalmus* spp. are highly specialised benthic diatom feeders, which are particularly abundant on well illuminated, intertidal deposits of mud (e.g. Yang et al., 2003). During this study the adults of these species were invariably found in well illuminated areas with no vegetation coverage, and on mud substrates. In Morib, due to recent artificial beach nourishment programme to mitigate coastal erosion, the sand was washed over and deposited on the mudflat and in the adjoining small mangrove forest (Seang, 2003). Here *Boleophthalmus* spp. are now absent, but they are still abundant along the mud banks of the neighbouring Morib river. Instead, in K. Selangor they are both present on river and creek mud banks, and on open mudflats. Nonetheless, the specialised feeding niche of *Boleophthalmus* spp. may only partly explain their habitat selection. The Malayan *Boleophthalmus* species present notched teeth in the lower mandible. We interpret this character as an adaptation to efficiently skim the mud surface while feeding on diatoms. Nonetheless, the Australian *B. birdsongi*, which also lives on exposed mudflats and creek mud banks, does not present notched teeth (Murdy, 1989). If this latter condition is assumed to be plesiomorphic, this would suggest that the adaptive process that led these species to herbivory occurred when these species were already present in these types of habitats. In this case, it seems that during their evolution *Boleophthalmus* species were ‘locked’ in habitats with more water availability by an increasing feeding specialization.

Pn. schlosseri was associated with intermediate levels of water availability (group B). During low tide, adults of *Pn. schlosseri* were found along the whole intertidal zone, but always at approx.≤ 1 m from the water edge. The described habitat distribution of this species is consistent with other reports from the Malay peninsula (adults: Berry, 1972; Sasekumar, 1974; Swennen et. al., 1995; Takita et al., 1999; young: Khaironizam and Norma-Rashid, 2005).

Two species of *Periophthalmus* were included in group A, inside mangrove forests and in more terrestrial conditions (*P. gracilis* and *P. novemradiatus*). Australian populations of *P.*

gracilis seem to live in more aquatic habitats, along creek banks and pioneer mangrove fringes (Milward, 1974). Takita et al. (1999) occasionally found *P. novemradiatus* in unvegetated, wet areas, on river banks or seashores; instead, they found numerous individuals of both *P. gracilis* and *P. novemradiatus* in a cleared area nearby an abandoned shrimp pond constructed in a mangrove forest. Swennen et al. (1955) found *P. novemradiatus* on the banks of narrow tidal ditches (= inlets) and creeks, fringed by vegetation and human waste. According to our observations, these habitats seem to be either marginal or artificial for these species, which during low tide were particularly abundant inside mangrove forests (*Rhizophora* and *Bruguiera* zone), and on the bottom of ephemeral tidal inlets. During flood tide, numerous individuals of *P. gracilis* and *P. novemradiatus* moved away from the water edge, aggregating along the banks of flooded ephemeral inlets.

P. chrysospilos was included by the AHC in group C, together with the more aquatic *Boleophthalmus* and *Scartelaos* spp. (**Fig. 4b**). Our habitat and behavioural descriptions are consistent with Berry (1972); Macnae (1968); Takita et al. (1999); and Khaironizam & Norma-Rashid (2005). The perching behaviour during high tide was also observed by Polunin (1972) in Singapore. To the best of our knowledge, Murdy (1986: … "a procession of *Ps. chrysospilos* following the tide") made the only other report of the shoaling and orientation behaviours towards the mudflat, that we observed during ebb tide.

The MCA and AHC described 2 very different mudskipper guildes with respect to habitat water availability (A, C). Since the used parameters increase in value from sea to land, this differentiation also corresponds to a spatial partition along the vertical intertidal gradient. Only larger individuals of *Pn. schlosseri* (B) are able to rapidly move between both zones, probably using the network of ephemeral inlets to move between them with tides. Other species may use these networks to migrate along the intertidal zone during ontogeny.

The analysis of co-occurrence between different locations was not statistically significant. In fact, the different number of species found in the studied locations probably reflects a lack of suitable habitats, determined by the gradual decrease of the extension of intertidal gradients from north to south in the Malacca Straits (Polgar, 2008). Instead, the significantly negative species co-occurrence found between sites and among species of groups A and B, suggests the presence of strong interspecific interactions. In particular, both pairs composed by *P. novemradiatus* or *P. gracilis* and the much larger *Pn. schlosseri* presented higher numbers of checkerbord units, accounting for the significantly high C-score (Gotelli and Entsminger, 2001). This is consistent with observations made also by Swennen et al. (1995), which noticed that this large predator rarely co-occurred with smaller mudskipper species or individuals. *Pn. schlosseri* is a known predator of mudskippers (Clayton, 1993; pers. obs.). A similar account of habitat segregation mediated by predator-prey interactions was reported by Nursall (1981) between the Australian *Pn. schlosseri* [= Pn. freycineti (Quoy and Gaimard, 1824)] and *P. expeditionum* (= *P. novaeguineaensis* Eggert, 1935). This may also allow to interpret both the dense aggregations of *P. gracilis* found around the burrow openings of *Pn. schlosseri* inside mangrove forests, and the peculiar, funnel-shaped burrow of this large predator (site S5). Since water availability was relatively limited inside forests, the large, flooded burrow of *Pn. schlosseri* may act as a trap, attracting small mudskippers looking for water. As also noted by Swennen et al. (1995), these large, funnel shaped burrows also acted as efficient traps for small marine visitors.

The lack of significant negative co-occurrence in among the species living in habitats with higher water availability may actually reflect a sample bias. In fact, we observed prey-predator interactions (*Pn. schlosseri* preying on *B. boddarti*); interspecific aggressive interactions; and the same partial spatial partition between *B. boddarti* and *B. dussumieri* (= *B. pectinirostris*) reported by Takita et al. (1999).

At genus level, the morphology and the physiology of the studied species are consistent with the systematic relationships described by Murdy's cladogram (1989; **EMS**, **S1**). The MCA and the AHC (**Fig. 4**) generally followed this same pattern. The most derived genera *Periophthalmus* and *Periophthalmodon* included species occurring in habitats with lower to intermediate levels of water availability (*P. novemradiatus*, *P. gracilis*, *Pn. schlosseri*); and both species of the less derived genus *Boleophthalmus* were found in habitats with higher water availability.

Nonetheless 1) the habitat water availability of the basal *Scartelaos* species was comparable to that one of *Boleophthalmus* spp.; and 2) the derived *P. chrysospilos*, the only species within group C which emerged during high tide (EM2: **Tab. 3**), was included in group C (**Fig. 4**). In the first case, it is possible that our quantification method had a lower resolution at lower levels of water availability, lumping together all aquatic species. Instead, the habitat of *P. chrysospilos* may represent an evolutionary reversal of some eco-ethological traits, or a less derived condition within the genus *Periophthalmus*. More probably, the adaptive significance of the EM2 condition is much higher than the different levels of habitat water availability.

Quantitative data on mudskipper distribution and abundance are still largely lacking. Mangrove and adjacent peritidal systems also present a wide range of structures (Robertson and Alongi, 1992). Therefore, different ecosystems should be systematically surveyed to gradually build a general model for the habitat distribution and differentiation of mudskipper species within a particular biogeographic region.

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Figures and tables

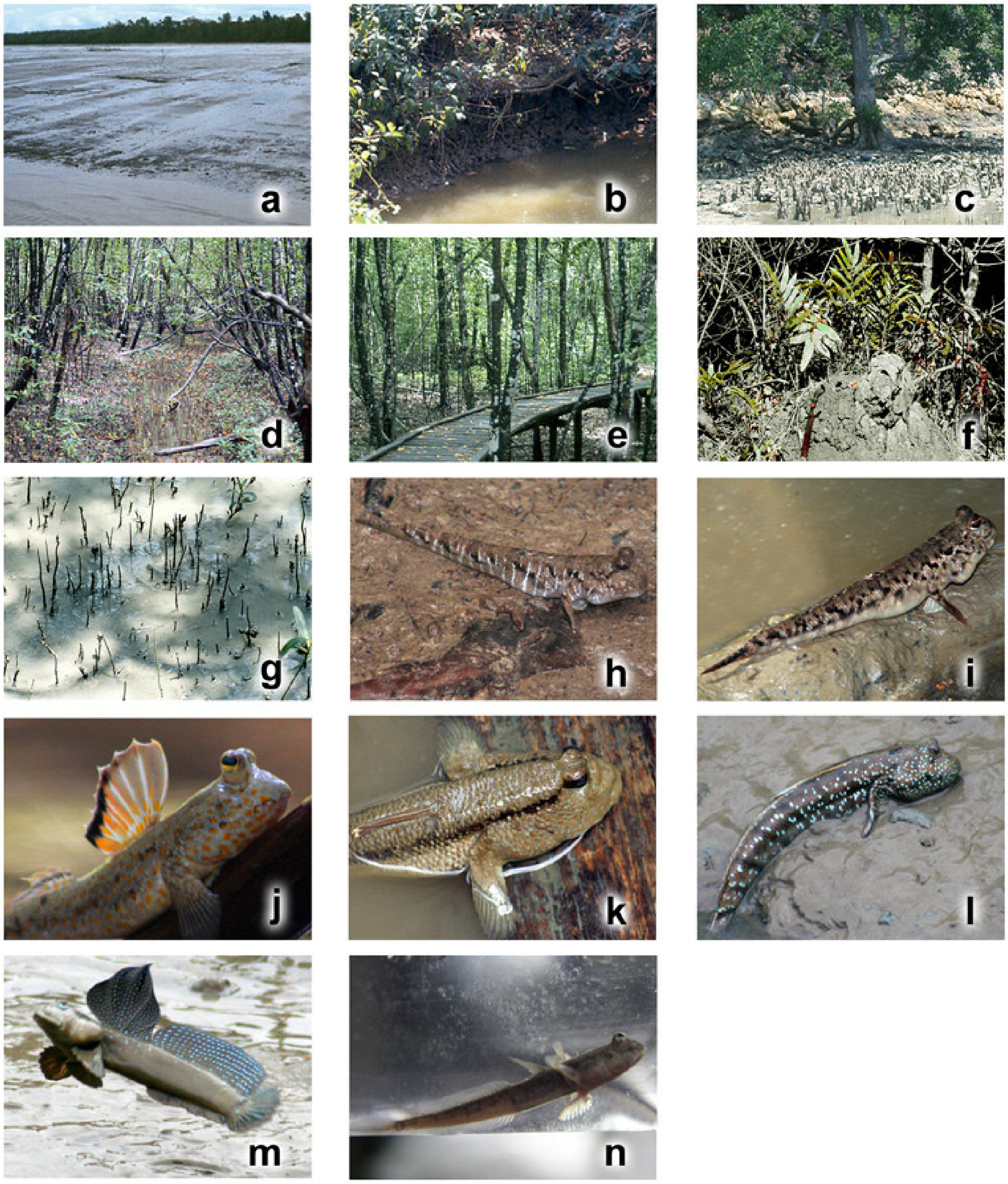


Figure 1

a-g: selected sites (**Tab. 3**) illustrating the described environmental ordinal variables (**Tab. 2**): density of vegetation coverage (VC), water bodies (WB), tidal influence (TI), and structural elements (SE); **h-m**: studied species. **a**: site K3, lower mudflat (1, 1, 1, 1, respectively); **b**: site S2, bank of an artificial brackish creek (2, 1, 1, 1); **c**: site R1, small stand of *Sonneratia alba*:

pioneer marine fringe (3, 2, 2, 3); **d**: site S5, tidal inlet during neap high tide (4, 3, 2, 4 – measured during low tide, when it was empty); **e**: site S4, *Rhizophora* and *Bruguiera* spp. forest (5, 4, 3, 4); **f**: site S3, *Acrostichum* sp. halophyte fern on top of a mud mound built by the crustacean *Thalassina anomala* (6, 4, 4, 4); **g**: site S7, pneumatophore zone of an *Avicennia alba* forest (1, 2, 2, 2); **h**: *P. gracilis*; **i**: *P. novemradiatus*; **j**: *P. chrysospilos*; **k**: *Pn. schlosseri*; **l**: *B. boddarti*; **m**: *B. pectinirostris*; **n**: *S. histophorus*: a young individual in a plastic bag. Photos **h-j**: Pulau Kukup, Johor, Malaysia; **k-m**: Kukup town jetty; **n**: Morib, Selangor, ibid.; photos by G. Polgar; photo **f**: courtesy of Prof. A. Sasekumar.

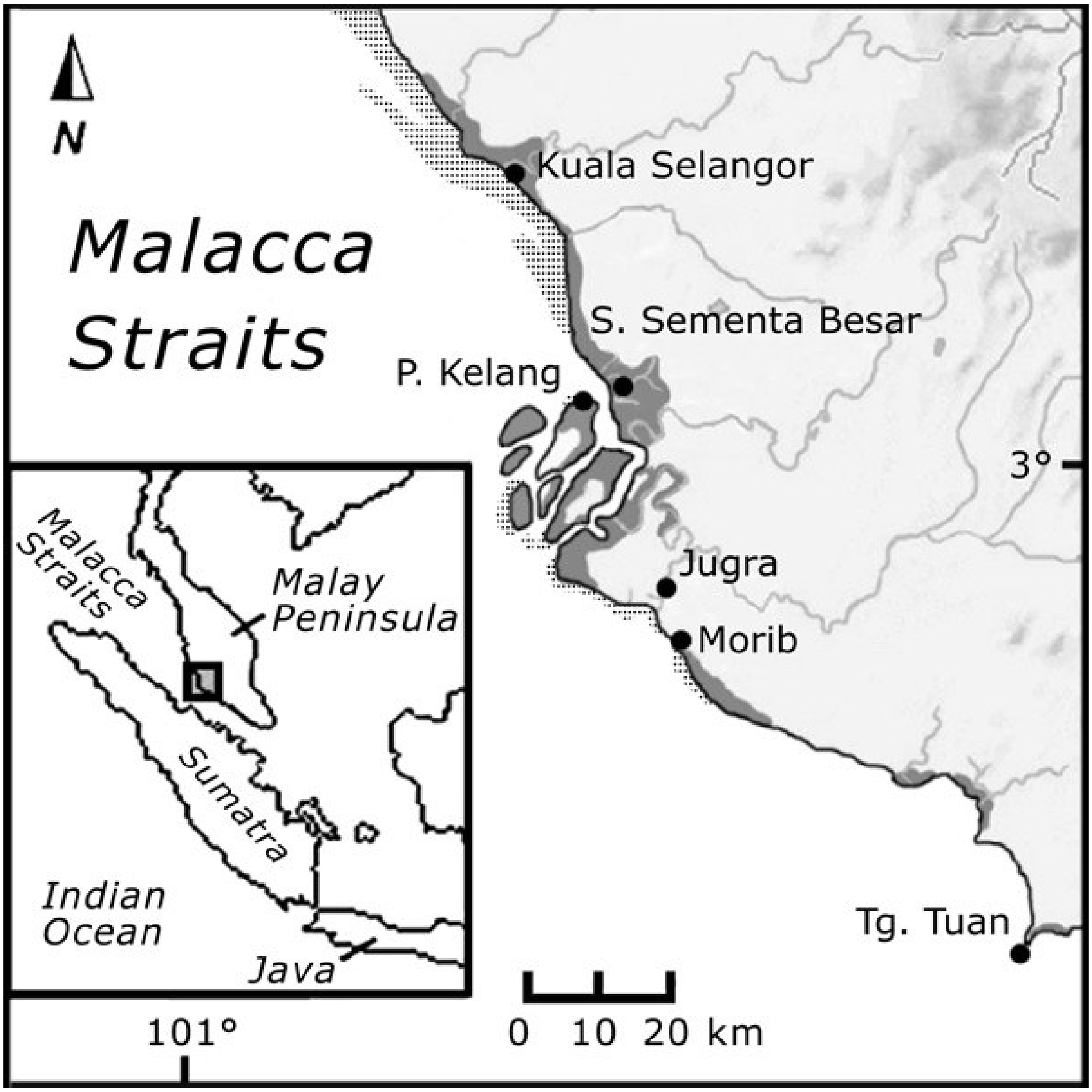


Figure 2

Study locations: western peninsular Malaysia; darker areas: mangrove forests; stippled areas: mudflats exposed during low tide; S.= Sungai (= River); P.= Pulau (= Island); Tg.= Tanjung (= Cape).

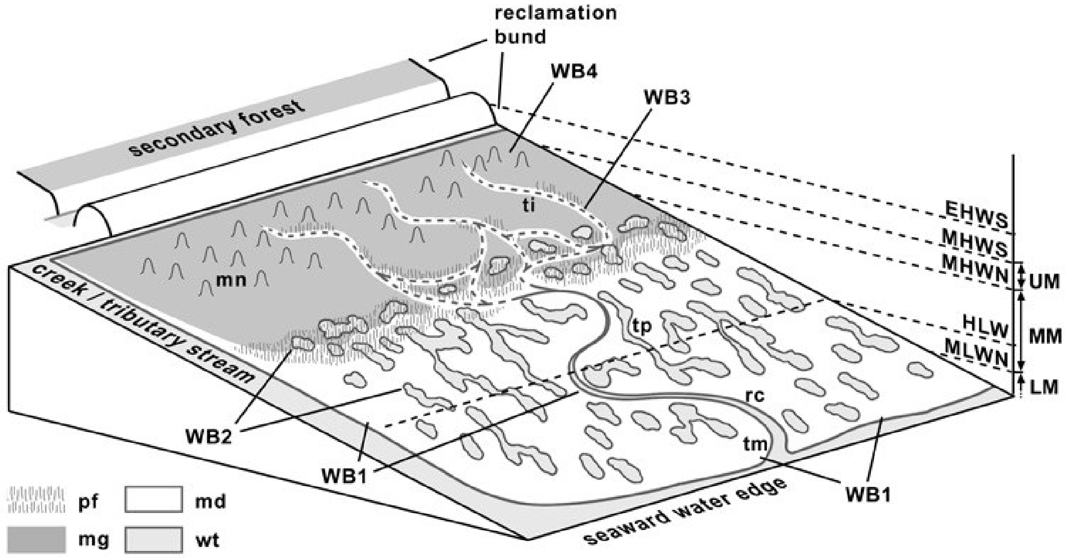


Figure 3

Block diagram of a Malayan mangrove and mudflat intertidal system during low tide. Due to land reclamation, the mangrove landward fringe is usually lost in Malayan systems: the high intertidal mangrove forest ends behind the reclamation bund (Sasekumar, 1980). If not cultivated, the reclaimed area is rapidly colonised by a freshwater secondary forest. Dark grey: mangrove area (mg); white: unvegetated mud (md); pale grey: water (wt). mn: mud mounds bult by crustaceans (*Sesarma* spp., *Thalassina anomala*); ti (dashed lines): ephemeral tidal inlets. The pioneer forest is indicated by pneumatophores (pf) of *Avicennia* and *Sonneratia* spp. Notice how these mangroves can follow the bottom of tidal inlets to the middle and high intertidal zone (Tomlinson, 1986; *pers. obs.*). Tide pools (tp) >1 m^2 are distributed from the pioneer forest to the lower mudflat. In the pioneer forest the interconnected network of tidal inlets (ti) flows into run-off channels (rc), which cross the mudflat and flow into the estuary (tidal mouths: tm). Creeks, rivers and run-off channels contain water (wt) during low tide. HLW= highest low water. UM= upper mudflat; MM= middle mudflat; LM= lower mudflat (Dyer *et al.*, 2000). See **Tab. 2** for other abbreviations.

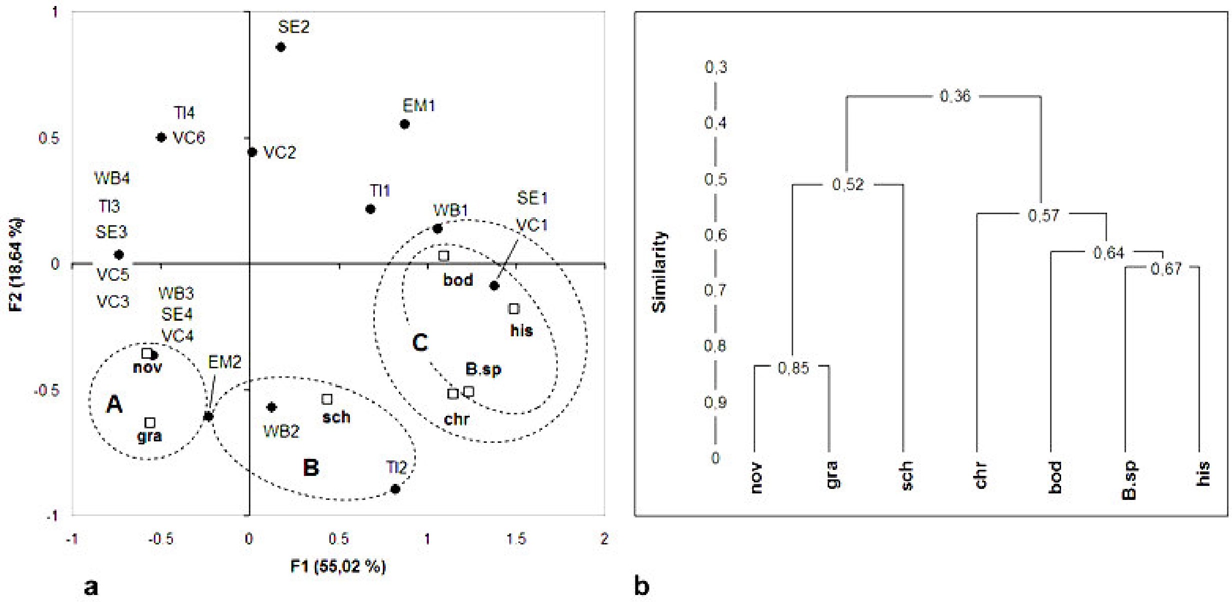


Figure 4

a: MCA: percentages: % variance explained by the F1 and F2 factorial axes; arbitrary dashed ellipses outline fish associations corresponding to AHC clusters (cut-off similarity value: 0.6), including closer points in the factorial space. Open squares: species; filled circles: environmental parameters; Species abbreviations use the first three letters of the specific name; other abbreviations as in **Tab. 2**. **b**: Agglomerative hierarchical clustering (AHC), applied to the same data matrix. Both analyses support three mudskipper associations: group A was found in more terrestrial (less aquatic) conditions and group C in more aquatic conditions; group B is intermediate. Within C, *P. chrysospilos* was found in the most terrestrial conditions.

Table 1. recorded mudskipper species (Oxudercinae: Periophthalmini) along the west coast of the Malay Peninsula. (**a**) Cantor (1849); (**b**) Koumans, (1953); (**c**) Berry (1972); (**d**) Murdy (1989); (**e**) Takita et al. (1999); (**f**) Khaironizam and Norma-Rashid (2002); (**g**) Khaironizam and Norma-Rashid (2003); (**h**) Polgar and Khaironizam, 2008; (**i**) this study. Abbreviations for generic names: *B.= Boleophthalmus*; *Pn.= Periophthalmodon*; *P.= Periophthalmus*; *Pd.= Pseudapocryptes*; *S.= Scartelaos*. [1]Species that were recorded, but not studied; [2]in Takita et al. (1999) *P. spilotus*= '*Periophthalmus* sp.'; [3]Ferraris, 1995.

species	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
B. boddarti (Pallas, 1770)	**x**	**x**	**x**	**x**	**x**			**x**	**x**
B. dussumieri Valenciénnes, 1837					**x**			**x**	
B. pectinirostris (Linnaeus, 1758)	**x**	**x**							**x**
Pn. schlosseri (Pallas, 1770)	**x**	**x**	**x**	**x**	**x**			**x**	**x**
Pn. septemradiatus (Hamilton, 1822)				**x**	**x**		**x**		**x**[1]
P. argentilineatus Valenciénnes, 1837			**x**	**x**				**x**	**x**[1]
P. chrysospilos Bleeker, 1852		**x**	**x**	**x**	**x**			**x**	**x**
P. gracilis Eggert, 1935				**x**	**x**			**x**	**x**
P. kalolo Lesson, 1830				**x**					
P. novemradiatus (Hamilton, 1822)				**x**	**x**			**x**	**x**
P. malaccensis Eggert, 1935		**x**							
P. spilotus Murdy and Takita, 1999					**x**[2]	**x**		**x**	**x**[1]
P. walailakae Darumas and Tantichodok, 2002								**x**	**x**[1]
Pd. elongatus (Cuvier, 1816)[3]	**x**	**x**		**x**	**x**				**x**[1]
Pd. borneensis (Bleeker, 1855)		**x**							
S. histophorus (Valenciénnes, 1837)	**x**	**x**	**x**	**x**	**x**				**x**

Table 2. Environmental terrestriality: definitions.

VC: density of vegetation coverage	
1	absence of vegetation coverage
2	patches of grass and bushes
3	open tree associations: pioneer mangroves (*Avicennia* or *Sonneratia* spp.)[a]
4	more open and depressed areas in VC5 (bottoms of inlets and gullies)[b]
5	*Rhizophora* and *Bruguiera* spp. zone[a]
6	*Bruguiera* spp. and halophytic ferns[a]
WB: water bodies	
1	flooded, larger waterways/basins, continuously activated by tides (<2 m from the water's edge of rivers, creeks, and sea)
2	smaller bodies of standing water, intermittently inundated by tides (tide pools >1 m^2)
3	waterways emptied by ebb tides (bottoms of tidal inlets and gullies)
4	animal burrows (only source of water)
TI: tidal influence	
1	area partially submerged by MLWN (Mean Low Water Neap)[c]
2	area submerged at least by MHWN (Mean High Water Neap)[d]
3	area submerged at least by MHWS (Mean High Water Spring)[e]
4	area submerged at least by EHWS (Extreme High Water Spring)[e]
SE: structural elements	
1	absence of structural elements
2	presence of pneumatophores[f]
3	presence of pneumatophores, trunks, and branches
4	presence of pneumatophores, aerial, prop and other types of roots, trees and vegetal debris (logs, branches, twigs, leaves, propagules)
EM: external medium during high tide	
1	fishes took shelter inside burrows when reached by the flood tide and remained under water during high tide
2	fishes came out of water when reached by the flood tide

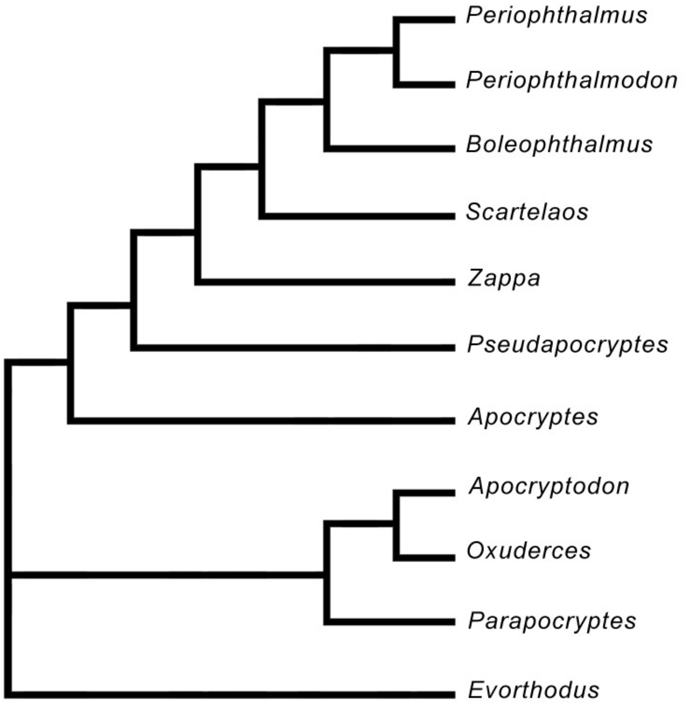
[a]Sasekumar, 1980; [b]Polgar, 2008; [c]e.g. lower unvegetated mudflats; [d]this datum generally corresponds to the pioneer forest marine fringe; [e]usually inside higher mangrove forests; [f]e.g. in front of the forest marine fringe (pneumatophore zone). VC, WB, TI and SE were measured during low tide, while EM was measured during flood tide.

Table 3. Species distribution and amount of environmental water

	Species distribution							**Environmental water**			
	EM= 1				EM= 2						
	chr	**nov**	**gra**	**sch**	**bod**	**pec**	**his**	VC	WB	TI	SE
S1 (1)	0	0	0	**1**	**1**	0	0	1	2	1	1
S2 (3)	0	0	0	**1**	0	0	0	2	1	1	1
S3 (2)	0	**1**	0	0	0	0	0	6	4	4	4
S4 (3)	0	**1**	0	0	0	0	0	5	4	3	4
S5 (5)	0	**1**	**1**	**1**	0	0	0	4	3	2	4
S6 (1)	0	**1**	0	0	0	0	0	5	4	3	4
S7 (1)	0	0	0	0	**1**	0	0	1	2	2	2
S8 (2)	0	0	0	0	0	**1**	0	1	2	2	1
S9 (1)	0	0	0	**1**	**1**	0	0	1	1	2	1
S10 (1)	**1**	0	0	0	**1**	0	0	1	1	1	1
K1 (1)	0	0	0	**1**	**1**	0	0	1	1	1	1
K2 (1)	0	**1**	**1**	0	0	0	0	5	4	3	4
K3 (1)	**1**	0	0	0	0	0	0	1	1	1	1
K4 (1)	0	0	0	**1**	**1**	0	0	1	1	1	1
B1 (1)	**1**	0	0	**1**	**1**	0	0	1	1	1	1
B2 (1)	0	**1**	**1**	0	0	0	0	5	4	3	4
J1 (1)	0	0	0	**1**	**1**	0	0	1	1	1	1
J2 (1)	0	0	0	**1**	0	0	0	1	1	1	1
J3 (1)	0	**1**	0	0	0	0	0	5	4	4	4
M1 (2)	0	0	**1**	0	0	0	0	3	2	2	3
M2 (2)	0	0	**1**	0	0	0	0	5	2	2	4
M3 (2)	0	0	**1**	0	0	0	0	3	4	3	4
M4 (1)	**1**	0	0	**1**	**1**	0	0	1	1	1	1
M5 (2)	**1**	0	0	0	0	0	**1**	1	1	2	1
R1 (1)	0	**1**	**1**	0	0	0	0	3	2	2	3
R2 (1)	0	0	**1**	0	0	0	0	5	4	3	4
nobs:	6	15	15	16	9	2	2	-	-	-	-

Species abbreviations: bod= *B. boddarti*; chr= *P. chrysospilos*; pec= *B. pectinirostris*; gra= *P. gracilis*; his= *S. histophorus*; nov= *P. novemradiatus*; sch= *Pn. schlosseri*; study sites and locations: S1-S10: Kuala Selangor; K1-K5: P. Kelang; B1-B2: Sg. Sementa Besar; J1-J3: Jugra; M1-M7: Morib; R1-R2: Tg. Tuan. In parentheses, number of replicate observations per site; nobs: total number of observations per species. Other abbreviations and definitions as in **Tab. 2**.

ESM – electronic supplementary material



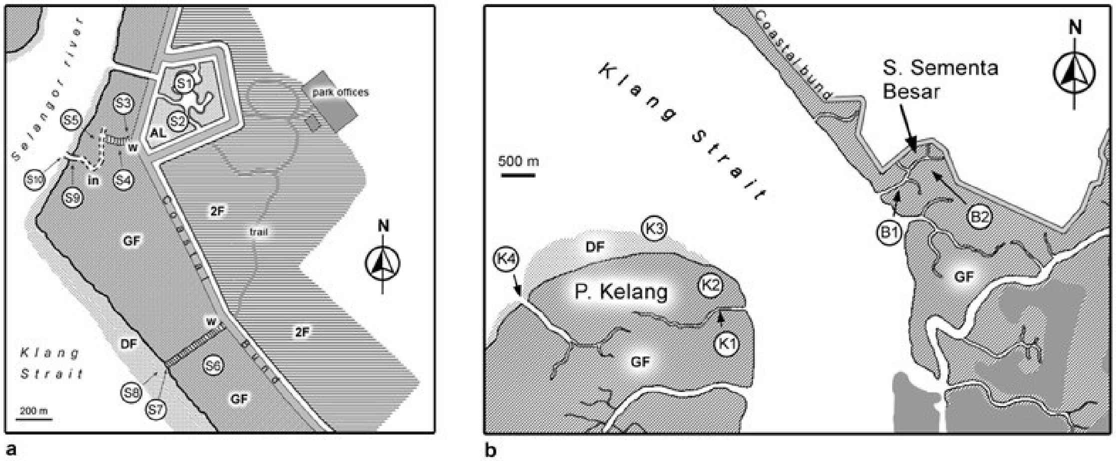
S1: cladogram of the Oxudercinae

Cladogram of the Oxudercinae (Murdy, 1989); the genus *Evorthodus* (Gobiidae: Gobionellinae) is a hypothetical sister group of the Oxudercinae. The subfamily includes two tribes: Periophthalmini, and Oxudercini; all other genera here studied are included in the tribe Periophthalmini. Species of derived genera (*Periophthalmus* and *Periophthalmodon*) present more extreme physiological and anatomical adaptations to terrestriality than basal genera (*Boleophthalmus* and *Scartelaos*).

S2: examined museum material and specimens deposited at the MSNG.

Boleophthalmus boddarti (Pallas, 1770): **15** specimens from 6 localities, Maharashtra, INDIA: Bombay (= Mumbai); Tamil Nadu, INDIA: Tranquebar (= Tharangambadi) and Madras (= Chennai); Khulna (Bagerhat), BANGLADESH: Mongla Upazila; Johor, MALAYSIA: Tanjung Piai; Selangor, MALAYSIA: Sementa, Sungai Sementa Besar; size range xx–xx mm SL: §ANSP 85017, **5** (84–99 mm SL), Bombay, *ibid.*, 1924; §ZMH 19369, **3** (35–53 mm SL) Bombay, *ibid.*, 1929; §ZMB 2145, **2** (128, 115 mm SL), syntypes of *Gobius striatus* Bloch & Schneider, 1801; Tranquebar, *ibid.*; §ANSP 77540, **1** (86 mm SL), Madras, *ibid.*, 1922; MSNG xxx [Bangladesh 14], **1** (xx mm SL), Mongla upazila, *ibid.*, 2008; MSNG xxx [TPB121106#05, 06], **2** (xx, yy mm SL), Tanjung Piai, *ibid.*, 2006; MSNG 54124 (108 mm SL), exposed creek's mud banks, Sungai Sementa Besar, *ibid.*, 30 August, 1996. *Boleophthalmus dussumieri* Valenciénnes, 1837: **7** specimens from 4 localities, Hormozgan, IRAN: Geru river and Bandar Khamir; KUWAIT: Khor Subiyah; Sind, PAKISTAN: Karachi; size range xx–xx mm SL: CMN FI 1979-0145, **1** (122 mm SL), Geru river, *ibid.*, 1976; MSNG xxxx [dusAbdoli_1], **1** (xx mm SL), Bandar Khamir, *ibid.*, 2005; MSNG xxxx [w-d150606KW], **2** (xx, yy mm SL), Khor Subiyah, *ibid.*; LACM 38125-6, **1** (xx mm SL), Sind, ibid.: Karachi, 1978; PMNH, uncat., **2** (116, 92 mm SL), Sind, *ibid.*, 2008. *Boleophthalmus pectinirostris* (Linnaeus, 1758): **17** specimens from 6 localities, Kyushu, JAPAN: Udo; Guandong, CHINA: Qi'ao Island; TAIWAN: Kaohsiung; Sumatra, INDONESIA: Pulau Bengkalis; Johor and Selangor, MALAYSIA: Tanjung Piai and Kampong Sungai Yu, respectively; size range xx–xx mm SL: NSMTP-33716, **4** (55–61 mm SL), Udo, *ibid.*, 1990; MSNG xxx [EB-590/CB1], **1** (xx mm SL), Qi'ao Island, *ibid.*, 2006; *NTM-S11173-001, **2** (143, 144 mm SL), Kaohsiung, *ibid.*, 1982; *NTM-S15524-001, **5** (77–112 mm SL), Pulau Bengkalis, *ibid.*, 1994; MSNG xxx [TPB121106#01, 02], **2** (xx, yy mm SL), Tanjung Piai, *ibid.*, 2006; *NSMT-P54457, **3** (161–175 mm SL), Kampong Sungai Yu, *ibid.*, 1997. *Periophthalmodon schlosseri* (Pallas, 1770): **1** specimen from 1 locality, Selangor, MALAYSIA: Kuala Selangor; MSNG 54125 (159 mm SL), forested high shore: *Bruguiera* spp. zone, 09 August, 1996. *Periophthalmodon septemradiatus* (Hamiton, 1822): **1** specimen from 1 locality, Selangor, MALAYSIA: Kampong Kuantan; MSNG xxxx [KS230906#02] (xx mm SL), small dike inside the village, 23 September, 2006. *Periophthalmus argentilineatus* Valenciénnes, 1837: **1** specimen from 1 locality, Negeri Sembilan, MALAYSIA: Tanjung Tuan; MSNG 54126 (46 mm SL), *Sonneratia alba* pioneer shore, 15 August, 1996. *Periophthalmus chrysospilos* Bleeker, 1852: **3** specimens from 2 localities, Selangor, MALAYSIA: Morib and Pulau Kelang; size range 65.2–74.3 mm SL: MSNG 54128, **2** (65, 74 mm SL), *Sonneratia alba* pioneer shore, Morib, *ibid.*, 17 August, 1996; MSNG 52024, **1** (74 mm SL), lower mudflat, northern coast of Pulau Kelang, *ibid.*, 30 August, 1996. *Periophthalmus gracilis* Eggert, 1935: **6** specimens from 4 localities, MALAYSIA, Selangor and Negeri Sembilan: Kuala Selangor; Pulau Kelang; Morib; and Tanjung Tuan; size range 27–40 mm SL: MSNG 54129, **2** (27, 30 mm SL), mixed mangrove forest, inlet network, Kuala Selangor, *ibid.*, 27 August, 1996; MSNG 54130, **1** (27 mm SL), mixed mangrove forest, Pulau Kelang, *ibid.*, 30 August, 1996; MSNG 54131, **1** (33 mm SL), *Rhizophora* spp. forest, Morib, *ibid.*, 17 August, 1996; MSNG 54132, **2** (31, 40 mm SL), *Sonneratia alba* pioneer shore and *Rhizophora* spp. forest, Tanjung Tuan, *ibid.*, 15 August, 1996. *Periophthalmus novemradiatus* (Hamilton, 1822): **6** specimens from 3 localities, MALAYSIA, Selangor and Negeri Sembilan: Kuala Selangor; Pulau Kelang; and Tanjung Tuan; size range 40–57 mm SL: MSNG 54133, **1** (44 mm SL), forested high shore, nearby the reclamation bund, Kuala Selangor, *ibid.*, 08 August, 1996; MSNG 54134, **2** (40, 57 mm SL), mixed mangrove forest, inlet network, Kuala Selangor, *ibid.*, 27 August, 1996; MSNG 54135, **2** (40, 49 mm SL), inside and in front of a *Rhizophora* sp. stand, Pulau Kelang, *ibid.*, 30 August, 1996; MSNG 54136, **1** (52 mm SL), *Sonneratia alba* pioneer shore, Tanjung Tuan, *ibid.*, 15 August, 1996. *Periophthalmus spilotus* Murdy and Takita, 1999: **1** specimen from 1 locality, Selangor, MALAYSIA: Sementa; MSNG xxx [S210906#01] (xx mm SL), forested high shore: *Bruguiera* spp. zone, 21 September, 2006. *Periophthalmus walailakae* Darumas and Tantichodok, 2002: **1** specimen from 1 locality, Selangor, MALAYSIA: Kuala Selangor; MSNG 51393 (109 mm SL), forested high shore: *Bruguiera* spp. zone, 8 August, 1996. *Pseudapocryptes elongatus* (Cuvier, 1816): **1** specimen from 1 locality, Selangor, MALAYSIA: Kuala Selangor; MSNG xxxx [KSA230906#04] (xx mm SL), small pool in the bund channel, along the mangrove landward fringe, 23 September, 2006. *Scartelaos histophorus* (Valenciénnes, 1837): **6** specimen from 3 localities, Selangor, MALAYSIA: Morib; and Johor, ibid., Muar and Parit Jawa; MSNG xxxx (xx mm SL), mixed tidal flat, 19 May, 2007; [§†]USNM 278437, [§†]USNM 00278453, [§†]USNM 00279354, southside of Muar river, Muar, ibid., 1985; [§†]USNM 00278312, Parit Jawa, ibid., 1985; [§†]USNM 279353, xxx.

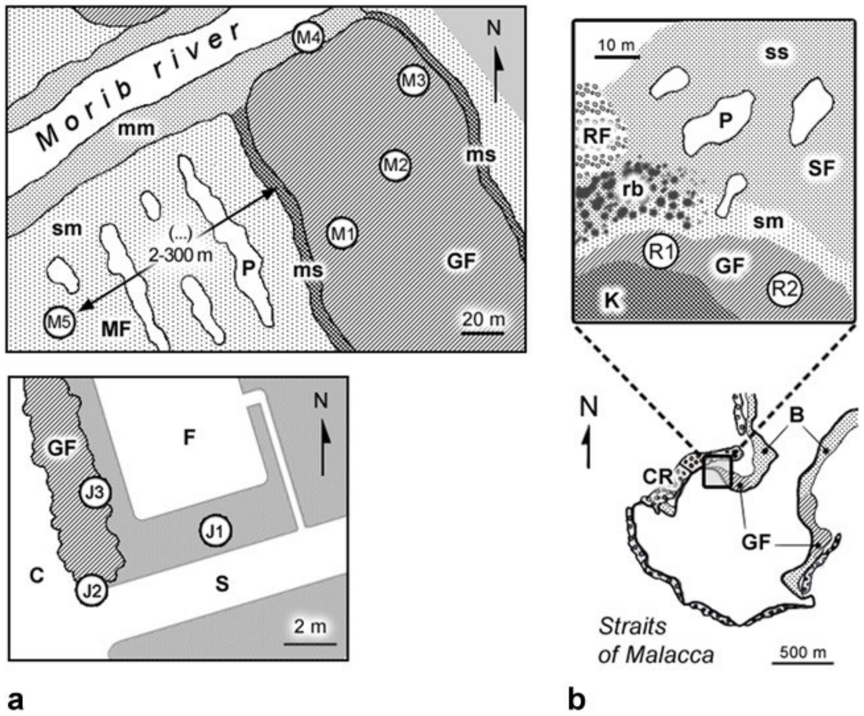
*according to the previous determiners, these specimens are *Boleophthalmus dussumieri* Valenciénnes, 1837; [§]these specimens were examined in high resolution digital photos; [†]according to the previous determiner, these specimens are *Scartelaos pectinirostris* (not valid).



S3: study sites (K. Selangor, P. Kelang and S. Sementa Besar)

a: Kuala Selangor (study sites: S1-S8: first transect; S9-S10: second transect). S3: landward fringe behind the reclamation bund; S4, S6: *Rhizophora* and *Bruguiera* spp. high forest; S5: bottom of an ephemeral tidal inlet inside the high forest; S7: *Avicennia* spp. pneumatophore zone in front of the pioneer mangrove marine fringe; S8: middle mudflat, a few meters from the forest marine fringe; S9: mud banks of the lower tract of a tidal inlet; S10: mud banks of the river Selangor; S1: artificial brackish lake; S2: artificial creek. **AL**: artificial lake; **GF**: mangrove forest; **DF**: exposed mudflat during low tide; **2F**: secondary forest; **w**: walking boards; **in**: tidal inlet; white: water and reclaimed areas.

b: Pulau Kelang (sites K1-K4). K1: mud banks of a tidal creek fringed by mangroves; K2: *Rhizophora* and *Bruguiera* spp. forest; K3: lower mudflat, nearby the water's edge; K4: tidal mouth of a run-off channel, nearby the water's edge. Sungai Sementa Besar (sites B1, B2). B1: mud banks of the lower tract of the creek; B2: high mangrove forest. Gray areas: urbanised areas (pier); acronyms and other graphic elements as in **a**.



S4: study sites (Morib and Tg. Tuan)

a *above*: Morib (sites M1-M5). M1: pioneer mangrove marine fringe of a *Sonneratia alba* forest; M2: *Rhizophora* spp. zone; M3: mixed zone with *Avicennia alba* and *Rhizophora mucronata*; M4: mud banks of the river Morib; M5: mixed tidal flat. **GF**: mangrove forest; **MF**: mixed flat; **P**: tide pools; **mm**: mud (river banks); **ms**: mud-sand; **sm**: sand-mud. *Below*: Jugra (sites J1-J3: adjacent to a shrimp farm). J1: mud banks of its waste channel; J2: mud banks of a nearby creek; J3: *Bruguiera* sp. mangrove stand along the creek banks. **C**: creek; **F**: shrimp farm; **GF**: mangrove stand; **S**: waste-channel.

b, *below*: Tanjung Tuan (Cape Rachado), topographic view; **B**: beach; **CR**: coral reef; **GF**: small mangrove stands. *Above*: Tanjung Tuan (sites R1, R2). R1: trees of *Sonneratia alba* in front of a reef flat; R2: stand of *Rhizophora mucronata*. **K**: rocks; **P**: tide pools; **RF**: reef flat; **SF**: sand flat; **rb**: boulders and pebbles; **sm**: sand-mud; **ss**: sand; other abbreviations as in **a**.

S5: matrix of species vs. environmental parameters. This matrix is directly obtained from **Tab. 3**, and was used for the MCA and AHC analyses.

	chr	**nov**	**gra**	**sch**	**bod**	**pec**	**his**
VC1	**1**	0	0	**1**	**1**	**1**	**1**
VC2	0	0	0	**1**	0	0	0
VC3	0	**1**	**1**	0	0	0	0
VC4	0	**1**	**1**	**1**	0	0	0
VC5	0	**1**	**1**	0	0	0	0
VC6	0	**1**	0	0	0	0	0
WB1	**1**	0	0	**1**	**1**	0	**1**
WB2	0	**1**	**1**	**1**	**1**	**1**	0
WB3	0	**1**	**1**	**1**	0	0	0
WB4	0	**1**	**1**	0	0	0	0
TI1	**1**	0	0	**1**	**1**	0	0
TI2	**1**	**1**	**1**	**1**	**1**	**1**	**1**
TI3	0	**1**	**1**	0	0	0	0
TI4	0	**1**	0	0	0	0	0
SE1	**1**	0	0	**1**	**1**	**1**	**1**
SE2	0	0	0	0	**1**	0	0
SE3	0	**1**	**1**	0	0	0	0
SE4	0	**1**	**1**	**1**	0	0	0
EM1	0	0	0	0	**1**	**1**	**1**
EM2	**1**	**1**	**1**	**1**	0	0	0

LEECHES ON MUDSKIPPERS: HOST-PARASITE INTERACTION AT THE WATER EDGE

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ABSTRACT: A host-parasite relationship was observed for the first time between a piscicolid leech and a species of amphibious goby (*Scartelaos tenuis*) from an intertidal mud flat in southern Iran. Morphological and molecular investigations assign the leech to *Zeylanicobdella arugamensis*. Of the 3 endemic and sympatric mudskipper species living in the Persian Gulf (*S. tenuis*, *Boleophthalmus dussumieri*, and *Periophthalmus waltoni*), leeches were only found on *S. tenuis* (prevalence and mean intensity= 71.4% and 2.3 ± 2.5, respectively), which is also the most aquatic. *S. tenuis* is not the largest species, but more leeches (≥4 leeches/host) were found on larger specimens (>12 cm Standard Length= SL). Nonetheless, in aquaria, leeches also attached on *P. waltoni*. This suggests an ecological partitioning of host-parasite complexes determined by hosts' habitat selection and/or leech limited resistance to air exposure.

Mudskippers are peculiar amphibious gobies (Gobiidae: Oxudercinae: Periophthalmini) which "are fully terrestrial activities for some portion of the daily cycle" (Murdy, 1989). They are distributed within tropical, subtropical and temperate zones, along the African Atlantic coasts and in the whole Indo-West-Pacific region, and live in estuaries and intertidal areas such as mangrove ecosystems and tidal mud flats. Where sympatric species occur, they are typically differentially distributed along the intertidal gradient (Milward, 1974; Nursall, 1981). Different genera show different degrees of eco-physiological adaptations to terrestriality (Clayton, 1993). This is typically paralleled by their habitat distribution (Takita et al., 1999). Moreover, the proposed phylogenetic cladogram for these genera suggests an increased degree of adaptation to terrestriality at each cladogenetic event (Murdy, 1989; Graham, 1997). Therefore, this monophyletic group offers a unique opportunity to compare the adaptations of closely related species during their eco-evolutionary transition from aquatic to semi-terrestrial habitats. Three endemic species are present in an area comprising the Persian Gulf, the Gulf of Oman, Pakistan, and the west coast of northern India up to Mumbai (Murdy, 1989). The 3 species are: *Scartelaos tenuis* (Day, 1876); *Boleophthalmus dussumieri* Valenciénnes, 1837; and *Periophthalmus waltoni* Koumans, 1955 (Fig. 1d-f).

[approx. location of Figure 1]

Iranian mudskippers are differentially distributed from more aquatic to more terrestrial habitats, respectively from *S.tenuis* to *B. dussumieri* to *P. waltoni* (Clayton, 1985). Very few available data confirm the presence of parasites in mudskippers either in nature (Pearse, 1932; Das, 1934; Mhaisen and Al-Maliki, 1996; Bandyopadhyay and Dash, 2001), or in aquaculture (Zhang and Hong, 2003). Mhaisen and Al-Maliki (1996) reported *Myxobolus pfeifferi* (Myxosporea or Myxozoa), *Diplozoon* sp. (Monogenea) and *Neochinorhynchus* sp. (Acanthocephala) on/in *Periophthalmus waltoni* in the Persian Gulf. In contrast, during observations made on specimens of several mudskipper species, i.e., *Boleophthalmus* sp., *Periophthalmodon* sp., *Periophthalmus* sp., and *Pseudapocryptes* sp., collected in Malaysia and Viet Nam, no parasites were found on their gills, suggesting a considerable resistance to aquatic parasites (G. Polgar and P. Galli, unpubl. obs.).

Here, we report the first record of leeches as ectoparasites on mudskippers (*Scartelaos tenuis*) from a tidal mud flat in southern Iran, together with laboratory observations in aquaria. Piscicolid leeches (Hirudinea: Piscicolidae) are widely distributed as fish ectoparasites in fresh- and brackish water of all continents except Antarctica, and in all oceans; many species occur in estuaries. The Piscicolidae was recently revised based on both morphological and molecular characteristics (Williams and Burreson, 2006). The fish leeches of the Indian Ocean were reviewed by Sanjeeva Raj et al. (1974). Only 3 species were reported from teleost fishes: *Malmiana* (=*Ottoniobdella*) *stellata* Moore, 1958; *Piscicola olivacea* Harding, 1920; and *Zeylanicobdella arugamensis* De Silva, 1963. Examination of the holotype of *M. stellata* from the Natal Museum shows it to be conspecific with *Z. arugamensis* (E. Burreson, unpubl. obs.). *Piscicola olivacea* has not been reported since the original description (Harding, 1920), although Sawyer (1986) transferred the species to the genus *Calliobdella*. More recently, Hayward (1997) reported *Z. arugamensis* (as *Z. stellata*) from Shark Bay, Western Australia.

We document here the occurrence in nature of an aquatic parasite only on the most aquatic of 3 sympatric and related species of amphibious hosts; this suggests the presence of differential selective pressures on host-parasite complexes with different degrees of adaptations to semiterrestrial life. We examine this hypothesis in the present report.

MATERIALS AND METHODS

Sampling

Live mudskippers were collected by hand on the mudflats of Bandar Khamir (Hormozgan Province, Iran; 26°56’40”N, 55°35’55”E: Fig. 1a), during 2 surveys in April and May 2007, and 3 surveys in August 2008 (Figs. 1b,c). Surveys were made within ± 2 hr around the predicted spring low tide at the nearest reference tidal station (Bandar Abbas, Admiralty EasyTide © UKHO). Sampling was made along three linear transects at three different levels of the intertidal gradient, i.e. at increasing horizontal distances from the sand dune to the creek (transects A-C: Fig. 1c; Table II). Individual fishes were placed into separate transparent plastic bags and transported alive to the laboratory. Fish were killed by placing them at -25°C for <1 hr. Mudskipper specimens were morphologically identified to species level by means of the available morphological keys (Murdy, 1989) and leeches were counted on each host; then mudskippers were measured to the nearest 0.1 cm (standard length; SL). Eight leech specimens were fixed and preserved in 96% ethanol for molecular analyses, while other specimens were fixed in 5% formalin and preserved in 60% ethanol for morphological studies.

In another experiment, several specimens of *S. tenuis*, *B. dussumieri*, and *P. waltoni* (Figs. 1d-f) were observed in a tank of 150 x 50 x 60 cm for a few days. The tank had been filled with a layer of mud and shallow water, taken from a tidal mud flat inhabited by mudskippers nearby the Hormozgan University, in Bandar Abbas, Iran. No filter system was used. They were maintained at room temperature and fed with algae and crab meat.

[approx. location of Figure 2]

A sample of *S. tenuis* was deposited in the Genoa Museum of Natural History, Genoa, Italy (MSNG XXX), while a sample of *Zeylanicobdella arugamensis*. was deposited as *Zeylanicobdella* sp. in the National Museum of Natural History of Washington, Washington D.C. (USNM 1114380), and in the Milan Civic Museum of Natural History, Milan, Italy (MSNM

Ar4765). DNA leech haplotype sequences were submitted to the EMBL (codes: FM208109; FM208110; FM208111).

Morphological analyses

Two individual leeches fixed in formalin were examined with a dissecting microscope. One of them was embedded in paraffin and serial sectioned transversely at 5 µm through the clitellum and anterior urosome following methods in Burreson and Kalman (2006).

Molecular analyses

DNA from 8 leeches was extracted and purified by using the DNAeasy® Tissue kit (QIAGEN, Qiagen Inc., Valencia, California) reagents. The concentration of the extracted solutions was adjusted to 3 ng/µl, and used directly for PCR amplification of a ~ 600 bp fragment of the COI region of mtDNA, using the primer LCO140 and HCO2198 (Folmer et al., 1994) and performing PCR reaction as detailed in Williams and Burreson (2006). The obtained templates were purified using standard procedure with NucleoSpin Extract (Macherey-Nagel, Germany). Direct sequencing was carried out using a 3730xl DNA analyzer in forward and reverse directions.

Sequences were aligned by using the software ClustalX 1.81 (Thompson et al., 1997) and adjusted by eye. The identification of haplotypes, polymorphisms, and parsimony informative sites were conducted with DnaSP 3.52 software (Rozas and Rozas, 1999). The species determination of the haplotypes was performed by aligning the obtained sequences with those found in the International Nucleotide Sequence Databases (INSD), using BLAST (Altschul et al., 1990) search engine. A set of closely related sequences from the international database was thus selected: 4 sequences of closely related taxa based on Williams and Burreson (2006) (DQ414344, *Z. arugamensis*; DQ414300, *Aestabdella abditovesiculata*; DQ414305, *Aestabdella leiostomi*; and DQ414334, *Pterobdella amara*); and a sequence of a species of Glossiphoniidae, used as outgroup (DQ995310, *Helobdella robusta*).

These sequences were used to build a phylogenetic hypothesis. The best evolutionary model fitting the dataset was selected by Modeltest 3.06 (Posada & Crandall, 1998). Two different approaches were then employed. A Maximum Parsimony (MP) analysis was performed using PAUP 4.0b10 (Swofford, 2002), building a strict consensus of the most parsimonious trees by TBR branch swapping. Bootstrap support values for nodes were computed (1,000 reps.). A Bayesian inference (BI) phylogenetic analysis was also performed (Huelsenbeck *et al*., 2001), using MrBayes (Huelsenbeck & Ronquist, 2001: 4 Markov chain, 300,000 generations; 100,000 generations were discarded as burn-in).

RESULTS

Eco-ethological observations

The marine leeches and their hosts were found at low tide on a tidal mud flat nearby Bandar Khamir, Hormozgan, Iran. The mudflat received fresh-water input from the small town nearby (Fig. 1b) and was separated from a salt flat by a sand dune which was parallel to the creek (Fig. 1c). Only a few stunted mangrove trees (*Avicennia marina*: Figs. 1b,c) were present above, or at the same level of a debris line, which indicates the approximate level of neap high waters (Fig. 1c). Above this line and nearby the sand dune (sand dune-transect A: Fig. 1c), the mud contained a sand fraction and was relatively compact. In this zone, *P. waltoni* and *B. dussumieri* were present, together with numerous ocypodid crabs. A large majority of *P. waltoni* with few *B. dussumieri* specimens were collected along transect A (Table II). Moving towards the sea (transects A-B: Fig. 1c), the sediment progressively became finer and the size and abundance of *B. dussumieri* increased, while the abundance of *P. waltoni* decreased; some individuals of *S. tenuis* were here present (Table II). Below the debris line, at about 150 m from the sand dune (transect C: Fig. 1c), *P. waltoni* and *B. dussumieri* rapidly declined in number as the mud became wetter. In this lower zone, the flat was covered by tide pools and there were areas of thixotropic mud. Here, *S. tenuis* was dominant (Table II). Piscicolid leeches were found only on specimens of *S. tenuis* (n= 7), with up to 7 leeches per individual (n= 16; prevalence: 71.4%; mean intensity: 2.3 ± 2.5). They were attached on the dorsum of the head, opercula, throat, and nearby the attachment of the anal and dorsal fins (Fig. 2). More leeches were found on larger *S. tenuis* (Fig.3), and the highest number of parasites (7) was found on the largest host (SL= 12.8 cm). No leeches were found on *B. dussumieri* (n= 22), and *P. waltoni* (n= 43), even though their size was comparable to *S. tenuis*; the largest captured mudskipper was a *B. dussumieri* (Table II).

In aquaria, the leeches attached also on *P. waltoni* (Fig. 2f), although they were never observed on individuals completely out of water. No movements of leeches were observed when attached to their host: they remained tightly attached with their caudal sucker, even after death by freezing.

[approx. location of Figure 3]

Leech morphology

Both leeches examined morphologically were 15 mm in total length, including suckers. The body is smooth, lacking gills, pulsatile vesicles, or papillae. Any pigmentation, including eyespots and ocelli, had totally faded in the formalin. The caudal sucker is eccentrically attached and moderate in size. The oral sucker is small. Histological sections revealed 5 pairs of testisacs, 2 pairs of mycetomes, and an extensive, ramifying, coelomic system. The sections confirmed the absence of pulsatile vesicles. The photographs (Fig. 2b) document dark brown or black pigmentation in the form of segmental transverse bands. There are also black pigment bands on the caudal sucker radiating from the sucker/urosome juncture to the margin of the sucker. The oral sucker has a transverse pigment band, and the crop of 1 leech contained blood from the host.

[approx. location of Figure 4]

Leech molecular analyses

Three haplotypes were identified (FM208109; FM208110; FM208111) among the 8 specimens examined, based on the alignment of 463 bp of the sequenced region. Variability among these sequences was relatively low (Table I), since only 3 polymorphic non-informative loci and 3 non-synonymous substitutions were detected. A BLAST search identified a COI sequence of *Z. arugamensis* (DQ414344) as the most similar one (94% identical), followed by those of *Pterobdella amara* (DQ414334, 91% identical), *Aestabdella abditovesiculata* (DQ414300, 91% identical) and *Aestabdella leiostomi* (DQ414305, 89% identical), respectively. These sequences were aligned with the new haplotypes and compared by means of p-distance (Table I). The new sequences showed a relevant divergence from the most similar taxon, namely *Z. arugamensis* (6.10 ± 0.10 sd). Nonetheless, the divergence between the Iranian leech and *Z. arugamensis* was determined by only 31 synonymous mutations, while the variability of all the piscicolid species is determined by 95 mutations, 11 of which were replacements.

[approx. location of Table I]

MP and BI phylogenetic hypotheses were highly congruent, and the trees had identical topologies (Fig. 4). Prior to analysis, Modeltest selected a GTR model (gamma correction= 0.3240). The new haplotypes formed a distinct clade, supported by a high bootstrap value and a-posteriori probability. Their affinity to the genus *Zeylanicobdella* was confirmed.

DISCUSSION

Identification of the piscicolid leech

The morphology of the leech found on *S. tenuis*, i.e. 5 pairs of testisacs, 2 pairs of mycetomes, an extensive coelomic system, shape of body and suckers, and black pigmentation in transverse bands, is consistent with *Zeylanicobdella arugamensis* De Silva, 1963. The molecular data demonstrate a relationship with the monophyletic clade comprised of *Zeylanicobdella*, *Aestabdella*, and *Pterobdella* (Williams and Burreson, 2006), with the sequences from the Iranian leech sister to *Zeylanicobdella arugamensis* in the phylogenetic analysis (Fig. 4). Nonetheless, the bootstrap support value for the relationship between the Iranian leech and *Z. arugamensis* (71) is not as high as the support value for the relationship between *A. leiostomi* and *P. amara* (83), two clearly distinct species. This might suggest that the Iranian leech is a distinct species; however, the p-distances, although perhaps greater than expected, are less for the Iranian leech/*Z. arugamensis* relationship than between *A. leiostomi* and *P. amara* (Table 1). *Zeylanicobdella arugamensis* is widely distributed in the Indian Ocean and throughout Indonesia. Variability in pigmentation pattern has been documented (De Silva and Fernando, 1965; Sanjeeva Raj et al., 1977), and is consistent with our observations, but little is known of the molecular sequence variability across the broad range of this leech. The only deposited sequence data are from a specimen collected in Borneo (Williams and Burreson, 2006), at the opposite end of the range from Iran. Until more is known about sequence variability of *Z. arugamensis* across its wide range, using multiple genes, we choose to identify the leech from *S. tenuis* as *Z. arugamensis*, based on morphology. The available molecular data are not inconsistent with that interpretation.

Zeylanicobdella arugamensis (=*Ottoniobdella stellata* Moore, 1958) was originally described from Sri Lanka, but is now known to be widely distributed throughout the Indian Ocean, Indonesia, the Philippines, and the Coral Sea, including the northern coast of Australia. It has been found as an ectoparasite on a wide range of demersal and benthic hosts from different fish families, namely Ariidae (*Tachysurus* [=*Arius*] *maculatus*); Bagridae (*Macrones* [=*Mystus*] *gulio*); Cichlidae (*Tilapia mossambica* [=*Oreochromis mossambicus*]); Gobiidae (*Glossogobius giuris*); Drepaneidae (*Drepane punctata*); Syngnathidae (*Hippocampus kuda*); Plotosidae (*Plotosus canius*); Serranidae (*Epinephelus coioides*); Sillaginidae (*Sillago* spp.); an unidentified marine eel; and an unidentified pufferfish (Moore, 1958; De Silva, 1963; De Silva and Fernando, 1965; Jayadev Babu, 1967; Sanjeeva Raj et al., 1977; Hayward, 1997; Cruz-Lacierda et al., 2000). This leech and its hosts typically live in estuarine brackish waters; the only record on a coral-reef species (*Epinephelus coioides*) is a leech infestation occurred after heavy rain in an aquaculture system (Cruz-Lacierda et al., 2000). This is consistent with our observations, since the tidal mudflat of Bandar Khamir receives continuous fresh-water inputs from the town nearby (Fig. 1b).

Aquatic parasites on semi-terrestrial hosts

This is the first record of a leech parasitizing a mudskipper. Intertidal aquatic conditions, e.g., burrows and tide pools, are particularly harsh at low tide, including rapid and wide fluctuations of temperature (Tytler and Vaughan, 1983), salinity (Sasekumar, 1994), and oxygen levels (Ishimatsu et al., 2000). Therefore, it can be reasonably argued that host-parasite complexes living in these systems are relatively highly specialized and co-adapted to such challenging conditions. In particular, mudskippers' ectoparasites should also be specifically adapted to peculiar host behaviors, such as air gulping (Graham, 1997), air phase maintenance in burrows (Ishimatsu et al., 1998; 2000), prolonged exposure to air (Sayer and Davenport, 1991), rapid and drastic temperature changes out of water (Tytler and Vaughan, 1983), and intense illumination. It is worth noting that while piscicolid leeches were only found on *S. tenuis* and in the most aquatic conditions in the field (transect C), they rapidly attached to other more amphibious species in aquaria (*P. waltoni*). This suggests that these parasites are able to tolerate only a limited exposure to air, being only present in the most aquatic environments inhabited by mudskippers. In fact, *S. tenuis* is the most aquatic of the 3 Iranian mudskipper species. It occurs in the lower intertidal zone (transects B, C: Fig. 1c), is frequently found half immersed in shallow water, and often occurs in areas of thixotropic mud.

Even though our sample of *S. tenuis* was relatively small, higher prevalence and mean intensity of parasites occurred during hotter months (April and June: 100%, 7.0 and 2.7, respectively; August: 33% and 0.3, respectively). *Z. arugamensis* presents demographic explosions in small water basins where the salinity drops during periods of intense rainfall (Cruz-Lacierda et al., 2000). In this region the rainy season is also particularly intense, when ephemeral torrents are formed (wadis: Fig. 1c). Moreover, mudskippers are known to hibernate during winter in the Persian Gulf, when the temperature drops below 10°C (Tytler & Vaughan, 1983). Therefore, seasonal changes of this host-parasite relationship are highly probable.

A strikingly similar system was studied by Goater (2000) in North America. He studied the differential occurrence of glossiphoniid leeches on sympatric species of plethodontid salamanders with different degrees of terrestriality. Also in this case, leeches parasitized more aquatic and larger hosts, and the system underwent drastic seasonal changes.

At present, no study is available on the coevolution of mudskippers and their parasite complexes. Nonetheless, host-parasites interactions seem to have played an important role during the eco-evolutionary transition of these gobies from aquatic to semi-terrestrial habitats. Incidentally, mudskippers are the only teleosts to be parasitized by terrestrial hematophagous insects (Ceratopogonidae, Culicidae; Okudo et al., 2004). Not unlike aquatic predators and intraspecific competitors (Sayer and Davenport, 1991; McNamara and Selden, 1993), parasites are likely to be a major selective force acting on the eco-evolutionary transition of amphibious fishes, both in water and on land.

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FIGURE 1. (a) Bandar Khamir (black arrow); (b) study site (W: alluvial conoids of seasonal turrents (wadis); U= urbanized areas; D= sand dune; S= sea; Ck= creek; Mn= stands of stunted mangroves; the area of study is inside the shaded rectangle with a hatched contour); (c) area of study (rd= road; dl= debris line deposited by the wave action during neap high waters; P= pier; the shaded rectangle with a hatched contour here shows the transect made during the surveys; other symbols as above); A, B, C: linear transects respectively made at 50, 100, 150 m from the sand dune, perpendicular to the water edge; maps drawn from satellite images (Google Earth Plus, v. 4.2). (d) *Scartelaos tenuis*; (e) *Boleophthalmus dussumieri*; (f) *Periophthalmus waltoni*; (d-f): freshly dead specimens from the area of study; the black bars are 10-mm long. Photos and drawings by G. Polgar.

FIGURE 2. *Scartelaos tenuis* and *Periophthalmus waltoni* with the piscicolid leech attached. (a) Head of freshly dead *Scartelaos tenuis*, dorsal view (bar: 5 mm); (b) the piscicolid leech (bar: 2 mm); (c) live leeches on a live specimen of *S. tenuis* inside a plastic bag, immediately after capture: left side of the head, ventro-lateral view; (d) head of a freshly dead *S. tenuis* with dead leeches still attached: lateral view (bar: 5 mm); (e) live *S. tenuis* in aquarium with leeches attached (black arrows): the fish is immersed in shallow water, emerging with only the eyes and

the head's dorsal portion; (f) live *P. waltoni* in aquarium with leeches attached. Photos by G. Polgar.

FIGURE 3. Number of leeches (nL) found on mudskippers (*S. tenuis*) of different size.

FIGURE 4. Phylogenetic relationships among the new mtDNA COI haplotypes and other homologous sequences. A Bayesian inference (BI) is illustrated; the topology of a maximum parsimony analysis (MP) was identical. Numbers at each nodes indicate a-posteriori probabilities obtained from the BI analysis (above) and bootstrap percentages relative to the MP analysis (below).

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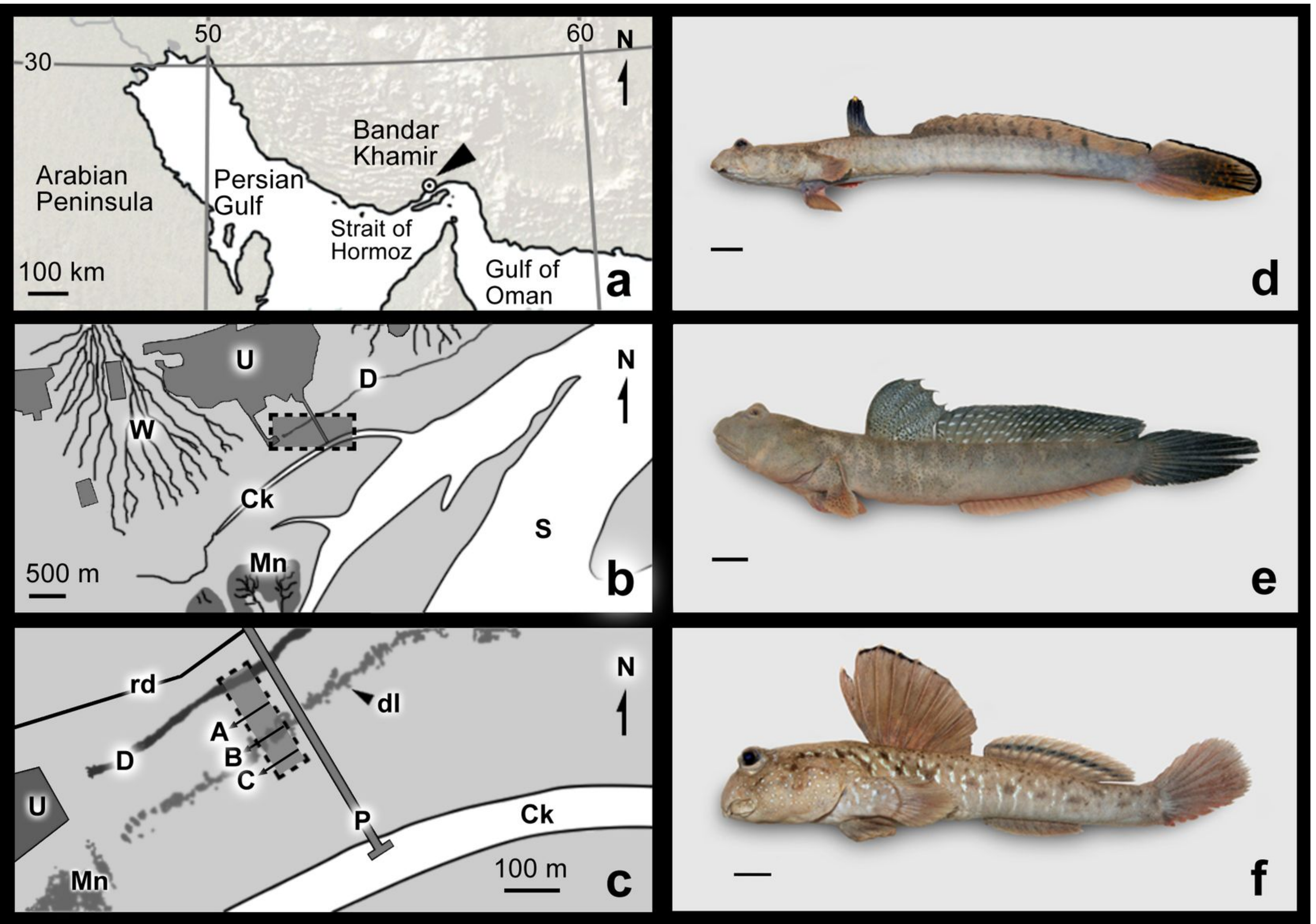
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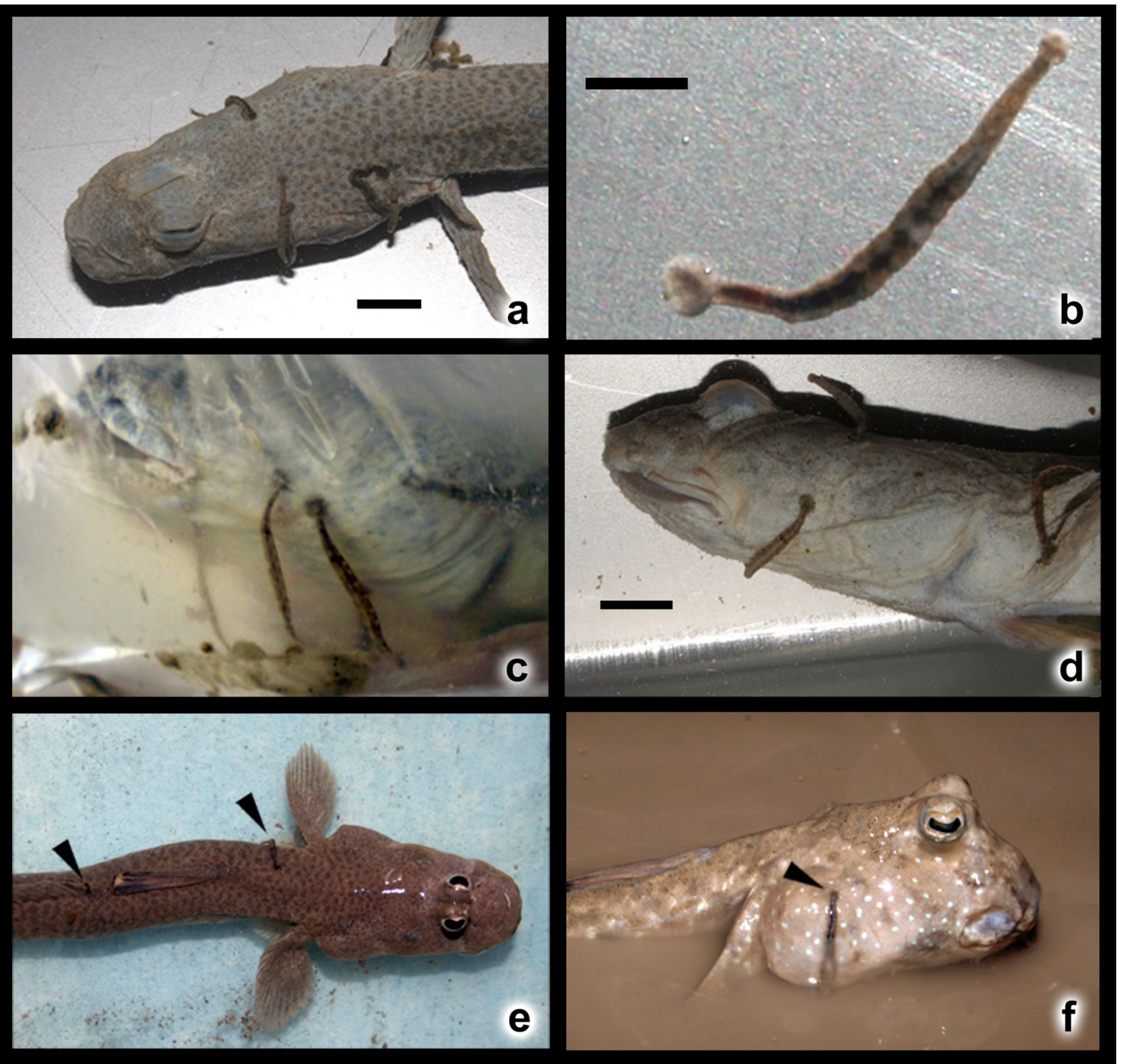
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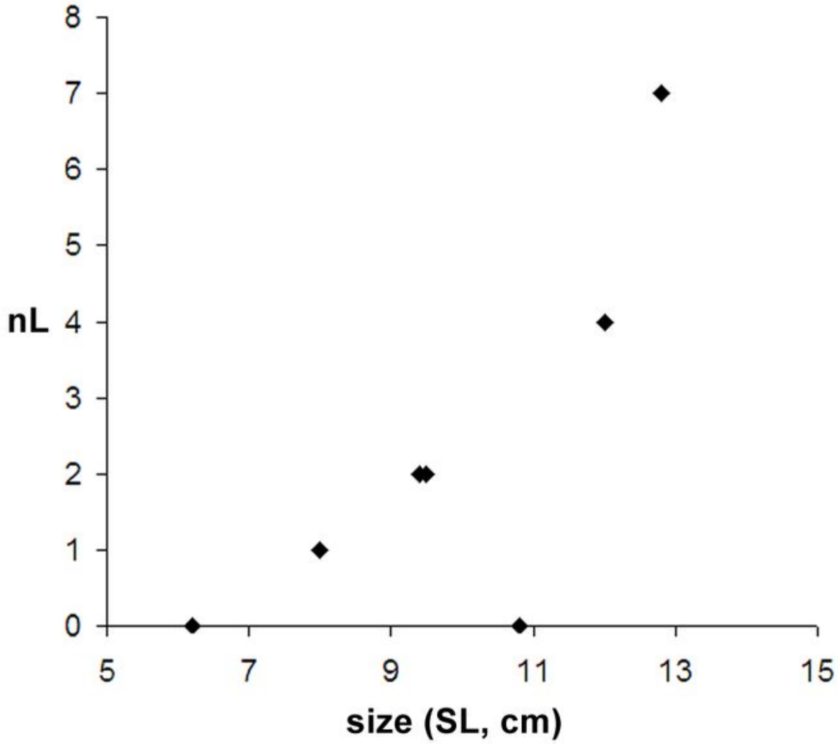
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size (SL, cm)

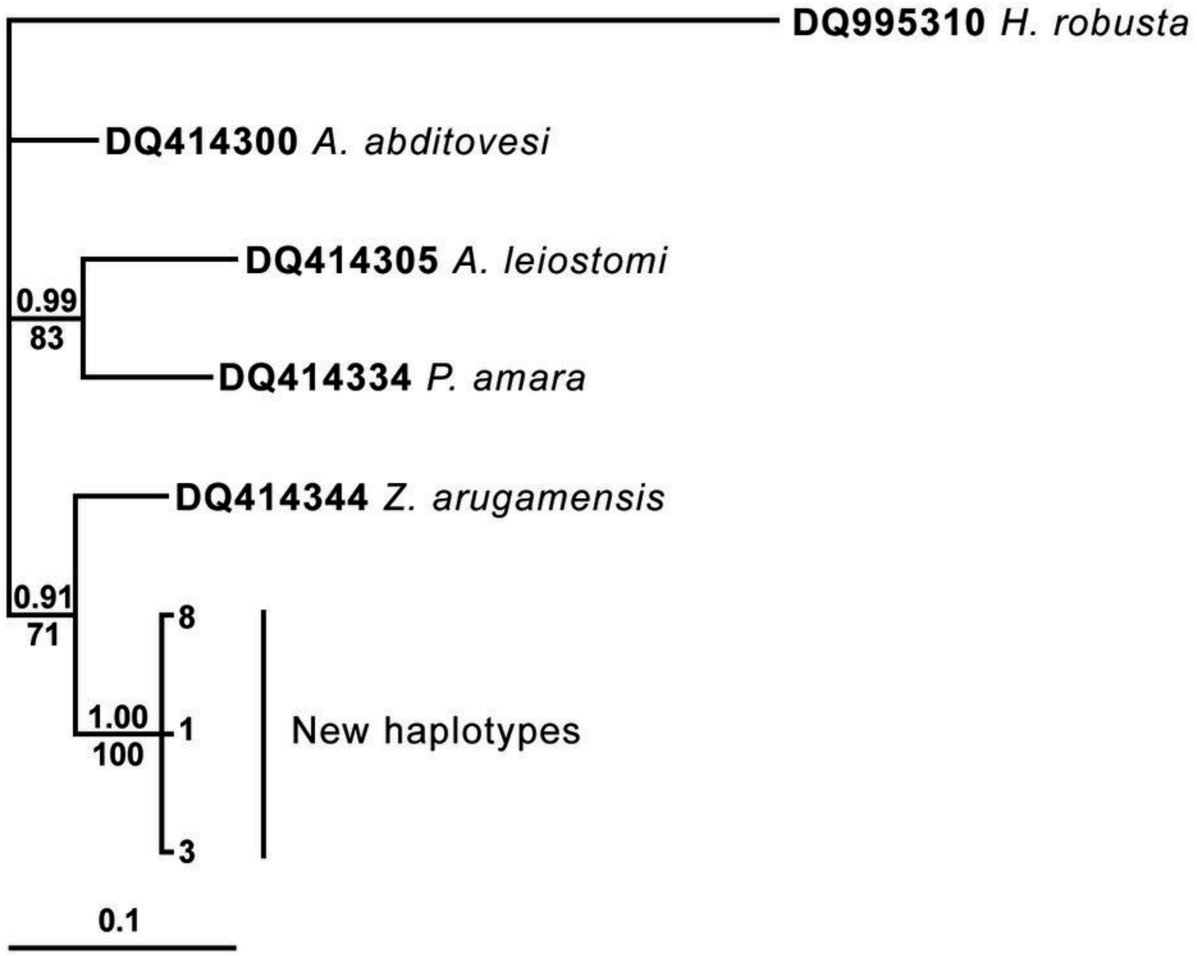
DQ995310 H. robusta
DQ414300 A. abditovesi
DQ414305 A. leiostomi
0.99
83
DQ414334 P. amara
DQ414344 Z. arugamensis
0.91
71
8
1.00
100
1
3
New haplotypes
0.1

Table 1. Matrix of p-distances (± 1 sd, when available) estimated among the piscicolid Iranian leech (iran) and the most closely related sequences available in INSD database, namely those of *Zeylanicobdella arugamensis* (DQ414344: arug), *Pterobdella amara* (DQ414334: amar), *Aestabdella abditovesiculata* (DQ414300: abdi) and *Aestabdella leiostomi* (DQ414305: leio). The intraspecific variability is reported only for the Iranian leeches.

	iran	arug	abdi	leio	amar
iran	0.16 ± 0.14				
arug	6.10 ± 0.10	-			
abdi	8.88 ± 0.07	8.42	-		
leio	10.21 ± 0.10	10.80	10.15	-	
amar	8.48 ± 0.10	10.38	9.72	9.29	-

Table 2. Mudskipper and leech sample size (n) and fish size (standard length, mean ± 1 s.d. in cm) at each linear transect A-C, as shown in Fig. 1c.

Species	A		B		C	
	length	n	length	n	length	n
Z. arugamensis	-	0	-	0	-	16
S. tenuis	-	0	10.8	1	6.2-12.8 (9.7 ± 2.5)	6
B. dussumieri	2.8-7.4 (5.5 ± 1.9)	4	4.5-15.3 (8.7 ± 3.3)	18	-	0
P. waltoni	3.9-11.2 (7.1 ± 1.7)	31	3.2-10.4 (7.8 ± 2.3)	12	-	0

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Abstract: During a survey in the region of the lower Fly river and delta, Papua New Guinea, several related species of amphibious gobies (Gobiidae: Oxudercinae) were found in environmental conditions with different degrees of aquaticity and salinity. A multivariate analysis of this correspondence was compared to the present phylogeny of this group. The results suggest that oxudercine gobies radiated from the sea, colonising semi-terrestrial and freshwater environments by differentiation and parallel adaptation of several taxa to subaerial conditions and lower salinities. Deltaic systems offer wide and gently sloping mudflats, that form wide ecological gradients from water to land. Here a whole array of gradually changing environmental conditions from aquatic to terrestrial conditions is realised. In these conditions, this group evolved highly eurytypic species,

capable to cross ecological barriers, and colonise freshwater environments at higher topographic levels. Recent paleoecological and paleontological studies also suggest that the ecology and adaptations of such species can be proposed as a convergent model to further our understanding of the vertebrate transition from water to land.

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Adaptation to semi-terrestrial and freshwater conditions in amphibious fishes of the lower Fly river and delta, Papua New Guinea. Estuaries as gateways from sea to land?

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Abstract

During a survey in the region of the lower Fly river and delta, Papua New Guinea, several related species of amphibious gobies (Gobiidae: Oxudercinae) were found in environmental conditions with different degrees of aquaticity and salinity. A multivariate analysis of this correspondence was compared to the present phylogeny of this group. The results suggest that oxudercine gobies radiated from the sea, colonising semi-terrestrial and freshwater environments by differentiation and parallel adaptation of several taxa to subaerial conditions and lower salinities. Deltaic systems offer wide and gently sloping mudflats, that form wide ecological gradients from water to land. Here a whole array of gradually changing environmental conditions from aquatic to terrestrial conditions is realised. In these conditions, this group evolved highly eurytypic species, capable to cross ecological barriers, and colonise freshwater environments at higher topographic levels. Recent paleoecological and paleontological studies also suggest that the ecology and adaptations of such species can be proposed as a convergent model to further our understanding of the vertebrate transition from water to land.

Introduction

Oxudercine gobies (Teleostei: Gobiidae: Oxudercinae; Hoese 1984; Murdy 1989) include highly amphibious species, or mudskippers, closely linked to tropical intertidal mudflats and mangrove forests (Clayton 1993; Graham 1997). Their geographical distribution spans from western Africa, to the whole Indo-West-Pacific region (Murdy 1989). Mudskippers occur sympatrically in several regions, being differentially distributed along the intertidal zone (e.g. Nursall 1981; Swennen et al 1995; Takita et al 1999). Several anatomical and physiological studies showed a gradual increase of the degree of adaptation to semi-terrestrial conditions from *Scartelaos* to *Boleophthalmus*, through *Periophthalmodon* and *Periophthalmus* species (e.g. Harris 1960; Milward 1974; Low et al 1990; Clayton 1993; Kok et al 1998; Ip et al 1990; Zhang et al 2000; 2003; Chew et al 2003). A comparison between this pattern and the present oxudercine phylogeny (Murdy 1989; **Fig. 1**) suggests that these genera evolved higher degrees of physiological adaptations to terrestriality at sequential cladogenetic events (Graham 1997).

Therefore, it should be possible to find a similar pattern also at ecological level, in the 'environmental terrestriality or aquaticity' of their habitats.

The southern Fly river and delta, Papua New Guinea (**Fig. 2**), maintains one of the richest, largest and pristine mangrove forests on earth, thanks to its peculiar oceanographic and sedimentological dynamics (Wolanski et al 1998). During several surveys along the banks and islands of the lower Fly river and delta, we recorded several oxudercine species, and the environmental conditions of their habitats. Few ordinal parameters were defined, which we assumed to be indexes of 'environmental aquaticity' (EA) in mudflat and mangrove ecosystems during low tide. We also recorded a differential distribution of mudskipper species along a salinity gradient of 250 km, from the delta to the lower tract of the river.

The aims of this study are: 1) to describe the geographical and habitat distribution of mudskipper species in this area; 2) to explore possible correspondences between their distribution and environmental conditions, with particular reference to terrestriality and salinity; and 3) to compare ecological traits with the present phylogenetic hypothesis, examining the correspondence between more derived taxa and more terrestrial conditions.

The possible implications of these studies for a proposed mudskipper model of the vertebrate transition from water to land are finally discussed.

Material and methods

Fieldwork

Observations inside mangrove forests and adjacent peritidal areas were made on feet by naked eye and binoculars, reaching sites by dinghy boat from field stations or research vessels. A reference collection was made, capturing fishes by hand net or digging them out of their burrows; a sample was deposited to the Genoa Natural History Civic Museum (MSNG). 17 sites were visited along the whole system, at increasing distances from the sea, from Sisikura Island (8°25.9' S, 143°36.8' E; site 13) to the Suki village (7°56.3’ S, 141°49.4’ E; site 6), up to about 250 km upriver (**Fig. 2**). In each site we surveyed and recorded the GPS position of several plots along the intertidal zone. Within each plot, environmental conditions were recorded and fish sampled by two researchers and 2-4 fishermen for approx. 0.5-1 hours.

High tide surface salinity and salinity of interstitial water in intertidal areas (Sasekumar 1994) were measured in the river and inside plots by a hand held refractometer. Measurements were categorised into three discrete intervals of salinities: SS1< 1 ppt; 2 ppt ≥SS2≥ 1 ppt; 10 ppt ≥SS3> 2 ppt; and 15 ppt ≥SS4> 10 ppt (Robertson et al 1991).

Tidal predictions were used to control for possible fish intertidal movements (Gibson 1999; Zander et al 1999). Observations were made under three different tidal conditions: 1) within ± 2 h to the low tide prediction (phase X); 2) in the absence of water bodies influenced by tidal action within the study plot (condition Y/A); and 3) in the presence of water bodies influenced by tidal action within the study plot (condition Y/P). Tidal predictions were obtained from the reference tidal station of Umuda (Admiralty EasyTide © UKHO), in the north-eastern delta (**Fig. 2**). Tidal action is prevalent in the delta, especially in the northern sector and during the south-east trade wind season (April-November: Wolanski et al 1988). Nonetheless, fluvial dynamics have an increasing influence upriver, and tidal predictions are less reliable at longer distances from the tidal station (E. Wolanski pers. comm.). No observations were made when raining.

We defined 'environmental aquaticity' (EA) as the total concentration of liquid, capillary and vapour environmental water present in a given area at ground level, and during low tide. Three ordinal parameters which are assumed to be indexes of EA in peritidal systems were then defined. Namely, vegetation coverage (VC); water bodies (WB); and structural elements (SE). For each parameter, higher values indicate less aquatic (more terrestrial) conditions.

Denser VC increasingly limit air movement and reduce evaporation rates at ground level (Macintosh 1977), determining higher levels of humidity. Six increasingly dense VC were recorded (Sarpedonti and Sasekumar 1996; Robertson et al 1991). Namely, 1) absence of vegetation coverage (VC1: **Fig. 3A**); 2) herbaceous and/or bushy vascular plants (VC2: **Fig. 3B**); 3) pioneer mangrove forests (VC3: **Fig. 3C**; group III in Robertson et al 1991); 4) bottom of ephemeral tidal inlets and gullies inside forests (VC4: **Fig. 3D**; Macnae 1968; Polgar 2008); 5) nypah mangrove forests (VC5: **Fig. 3E**; group II in Robertson et al 1991); and 6) transitions from lowland rainforest to freshwater swamps (VC6: **Fig. 3F**; these forest present dense undergrowths; representative arboreal species are *Pandanus* sp., and *Metroxylon sagu*).

Water bodies (WB) are hydrogeomorphic and biogenic structures which are filled with water during low tide, acting as sources of liquid water for mudskippers. Within a given area, larger and more persistent WB both determine more humid conditions at ground level, and increase water availability. Four increasingly small and ephemeral WB were recorded inside study plots. Namely, 1) banks of rivers/creeks and non-ephemeral run-off channels, at <1 m from the water edge (WB1: **Fig. 3G**); 2) tide pools approx.> 1 m^2 wide (WB2: **Fig. 3H**); 3) smaller pools (< 1 m^2) and bottoms of ephemeral tidal inlets and gullies (WB3: **Fig. 3I**); 4) absence of WB, or presence of water only in burrows of fishes and/or macroinvertebrates (WB4: **Fig. 3J**).

Structural elements (SE) are biotic and abiotic objects whose size is comparable to mudskippers (total length: 4-25 cm). SE both limit air movement at ground level, and increase substrate heterogeneity, thus increasing the concentration of capillary water. They can vary in size and density, according to the type of vegetation and sedimentological conditions. Nine increasing levels of substrate heterogeneity were recorded, by visual estimate of SE density and classification of SE types. Namely, 1) absence of structural elements (SE1: **Fig. 3K**); 2) smaller (e.g. leafs and twigs) and sparse vegetal debris on vegetated banks of creeks/rivers (SE2: **Fig. 3L**); 3) pneumatophore zones of *Sonneratia* spp. (SE3: **Fig. 3M**); 4) pneumatophores, trees and logs inside pioneer mangrove forests (SE4: **Fig. 3N**); 5) more heterogeneous (e.g. leafs, twigs, fronds, logs) and denser vegetal debris on vegetated banks of creeks/rivers (SE5: **Fig. 3O**); 6) trees, roots (e.g. pneumatophores, prop roots, knee roots, aerial roots) and more heterogeneous vegetal debris inside higher mangrove forests (SE6: **Fig. 3P**); 7) undercuts with exposed tree roots and heterogeneous vegetal debris along creek/river erosive banks (SE7: **Fig. 3Q**); 8) trees, various types of roots, bushes, litter, peat and heterogeneous vegetal debris inside freshwater swamps (SE8: **Fig. 3R**); and 9) buildups of logs, branches and heterogeneous vegetal debris along creek/river banks, or in the upper tract of creeks and inlets (SE9: **Fig. 3D**,**S**).

Finally, to control for possible ecological partitioning of different size classes (Clayton 1993), the individuals of each species were separated into two size classes, namely “adults”: total length (TL)> 50% of the maximum recorded TL (= TL_{max}); and “young and juveniles”: TL≤ 50% TL_{max}.

Data analysis: species vs. environmental conditions

Multiple correspondence (or homogeneity) analysis (MCA: Jobson 1992; XLSTAT 7.5.3 © Addinsoft) was used to explore the correspondence between species (modalities) and ordinal environmental parameters (observations). To further support the association of the species in the factorial space, we used agglomerative hierarchical cluster analysis with strong linkage aggregation method (AHC: XLSTAT 7.5.3 © Addinsoft), calculating nodes’ similarity by the Jaccard index (Johnson and Wichern 1992).

In a first analysis, we explored the correspondence between species and environmental aquaticity (parameters VC, WB and SE). In this case, only data collected in appropriate tidal conditions were used (phase X or Y/A conditions, hereafter indicated as “low tide”).

Since salinity (SS) may not be related to EA, we analysed it separately. In this case modalities would be more than observations, thus only the AHC was used. In this case we analysed the whole dataset, to account for variation during the tidal cycle.

A jackknife protocol was finally applied to all analyses by alternatively eliminating each observation in the data matrix, to search for influential observations that could bias the results (Ellison and Gotelli 2004).

Results

Species

Nine species were found (**Fig. 4**), namely *Oxuderces wirzi* (Koumans, 1938); *Zappa confluentus* (Roberts, 1978); *Scartelaos histophorus* (Valenciénnes, 1837); *Boleophthalmus caeruleomaculatus* McCulloch and Waite, 1918; *Periophthalmodon freycineti* (Quoy and Gaimard, 1824); *Periophthalmus darwini* Larson and Takita, 2004; *Periophthalmus murdyi* Larson and Takita, 2004; *Periophthalmus novaeguineaensis* Eggert, 1935; and *Periophthalmus weberi* Eggert, 1935. *P. darwini* and *P. murdyi* are first records for Papua New Guinea. Two more species, *Boleophthalmus* sp. and *Periophthalmus* sp., were not corresponding to any taxonomic key (Murdy 1989; Larson and Takita 2004) and are currently under description.

The following abbreviations will be used for generic names: *B.= Boleophthalmus*; *Pn.= Periophthalmodon*; *P.= Periophthalmus*; *S.= Scartelaos*; *O.= Oxuderces*; *Z.= Zappa*.

Environmental Aquaticity

In the MCA of species vs. EA, the first two factorial axes (F1, F2) accounted for 70.6% of total variance (**Fig. 5a**). With the only exception of young *Boleophthalmus* sp., the test values of the modalities of F1 and F2 were all significant (α= 0.05, two-tailed test). The fourth quadrant contains the lowest EA values (i.e. the most aquatic conditions: VC1, SE1, WB1); the second and third quadrants contain the highest EA values (i.e. the least aquatic or most terrestrial conditions, e.g. VC6, SE9, WB4), and some intermediate values (VC2, SE2,3); the first quadrant contains only intermediate values (VC3, SE4, WB2,3).

Five homogeneous associations were also supported by the AHC:

1. **W** (node's similarity= 0.68) – <u>more aquatic conditions</u>: young and adults of *B. caeruleomaculatus*; young and adults of *O. wirzi*; young of *Z. confluentus*; and adults of *Pn. freycineti*. These fishes were found on open mudflats, or on unshaded mud banks of creeks/rivers without vegetal debris (VC1, SE1); they were found nearby the water edge of large water bodies, or of pools (WB1-3)
2. **I** (node's similarity= 0.79) – <u>intermediate EA conditions (1)</u>: young and adults of *S. histophorus*; young and adults of *P. murdyi*; adults of *Z. confluentus*; adults of *Boleophthalmus* sp.; and adults of *P. novaeguineaensis*. These fishes were found in a wider range of conditions than **W**: they were also found on unshaded mud banks with vegetal debris (SE2); in pneumatophore zones (SE3); and inside pioneer mangrove forests (VC3, SE4); as in **W**, they were found nearby the water edge of creeks/rivers bodies, or of pools (WB1-3)
3. **WI** (node's similarity= 0.71) – <u>intermediate EA conditions (2)</u>: young of *Boleophthalmus* sp. and adults of *P. darwini* plot in an intermediate position between groups **W** and **I**; the AHC relates this group to group **W** (node's similarity= 0.59). These mudskippers were found in more aquatic conditions (VC1, SE1), but also inside higher forested areas (VC4,5, SE6); they were never found nearby larger tide pools (WB1,3)
4. **T** (node's similarity= 0.72) – <u>more terrestrial conditions</u>: young of *P. weberi* and young and adults of *Periophthalmus* sp. were found on shaded river banks with vegetal debris (VC2, SE5); in pioneer mangrove forests (VC3, SE4); on the bottom of ephemeral waterways in forested areas (VC4, SE6); and inside nypah forests (VC5, SE6). The young of *P. weberi* were also found among buildups of

logs and branches (SE9). They were found in all conditions of water availability (WB1-4)

5. **V** – <u>extremely variable conditions</u>: adults of *P. weberi* were found in a wide range of conditions, from shaded mud banks of creeks with debris (VC1,2, SE5,9); to deforested areas with shrubs and grasses (VC2, SE2); to higher forested areas (VC4,5, SE6,9). They were also found in habitats where no other mudskipper were present, such as freshwater swamps (VC6, SE8); floating buildups of logs in the upper tract of creeks (VC1, SE9); and undercuts of erosive banks (VC1,2, SE7). They were found in almost all conditions of water availability, larger tide pools excluded (WB1,3,4).

Compared to adults, juveniles of *Z. confluentus* were found in the most aquatic conditions during low tide, aggregating nearby the water edge (VC1, SE1, WB1). Young of *P. weberi* were not found in the most terrestrial conditions. A more evident ecological partition was observed in *Boleophthalmus* sp., where young were found in more terrestrial conditions (SE6, VC4,5) than adults (**table 1**). No relevant differences were found between young and adults of other species. The young/juveniles of *P. novaeguineaensis*, *Pn. freycineti* and *P. darwini* were not observed.

The jackknifing highlighted the importance of some environmental conditions for the definition of groups. The most important changes were caused by the elimination of either SE6, WB3, or VC4 (associated with bottoms of ephemeral inlets and higher forested areas), which caused a splitting of group **W**; and by the elimination of either SE4, or VC3 (pioneer mangrove forests), which caused the fusion of groups **W** and **I**.

Most changes involved, as expected, the modalities of intermediate groups, such as **WI** and **I**.

Under Y/P conditions, adults and young of *P. weberi*, and adults of *P. darwini*, *P. murdyi*, and *Periophthalmus* sp. were found in more aquatic conditions, nearby the water edge of tidal creeks (e.g. SE1, WB1, **table 1**). Therefore, these species seem to perform intertidal movements (Gibson 1999; Zander et al 1999), moving away from the water during flood tide, and waiting for the ebb tide along the water edge. During low tide, these species were also found at considerable distance from the the water edge.

Salinity

Surface salinity measurements made during high tides were in general accordance with the isopleths drawn by Robertson et al (1991: **Fig. 2**); nonetheless, higher values were recorded along a transect made on the banks of Purutu channel, both at lower and higher intertidal levels (sites PU023, PU027: **table 1**). Salinities of 0 ppt were recorded in all locations upriver of Tapila (**Fig. 2**).

The AHC supported four homogeneous mudskipper associations (**Fig. 5b**):

1. **FW** (node's similarity= 1.00) – <u>freshwater</u> (SS1): adults and young of *Z. confluentus*
2. **Sl** (node's similarity= 0.87) – <u>lower salinity</u> (SS2,3): adults of *Boleophthalmus* sp., adults and young of *S. histophorus*, adults of *Pn. freycineti* and adults of *P. murdyi*
3. **SS** (node's similarity= 0.89) – <u>higher salinity</u> (SS3,4): all other species/size classes, *P. weberi* excluded

4. **SV** (node's similarity= 1.00) – highly variable salinity (SS1,3,4): adults and young of *P. weberi*.

P. darwini was found only in SS3 conditions.

The jackknifing showed relevant changes when less saline conditions are eliminated (SS1,2), determining a fusion of cluster **SS** with either **SV**, or **SI**.

No significant correlation was found between salinity (SS) and any measure of environmental aquaticity among sites (VC, WB, SE; Spearman's D, Kendall's tau: p> 0.05).

Discussion

Environmental aquaticity and mudskipper evolution

The hypothesis of a sequential origin of increasing degrees of adaptation to terrestriality in more derived species (**Fig. 1**: *Periophthalmodon* + *Periophthalmus*> *Boleophthalmus*> *Scartelaos*> *Zappa*) is only partly supported by our analyses (**Fig. 5a**). In fact, species found in more terrestrial conditions are included in the more derived genus *Periophthalmus* (*P. weberi*, *Periophthalmus* sp.). Nonetheless, several congeneric species and the sister genus *Periophthalmodon*, were also present in more aquatic or intermediate conditions (*Pn. freycineti*; *P. murdyi*; *P. darwini*; *P. novaeguineaensis*); at the same time, while species of less derived genera were found in more aquatic conditions (e.g. *B. caeruleomaculatus*), other ones were found in intermediate conditions (*Boleophthalmus* sp., *S. histophorus*; *Z. confluentus*); the only representative of the tribe Oxudercini, *O. wirzi*, was only found in more aquatic conditions. Therefore, the present phylogeny seemingly describes several parallel pathways to terrestriality within each genus. Eventually, the most terrestrial environments were colonised by some species of the most derived genera.

Estuarine salinity gradients and the colonisation of more terrestrial habitats

Salinity in the Fly delta and Gulf of Papua is affected by the interplay of intense river discharge and seasonal winds (Wolanski et al 1995, 1998). Within the delta, high tide surface salinity is highly variable with time, especially around spring tides, when it can nearly double its value in a few days. Even more drastic fluctuations occur during the whole daily tidal cycle, from nearly full freshwater, to full seawater (Wolanski et al 1998). Conditions in the intertidal zone and during low tide are reasonably even more variable, due to the synergistic effects of evaporation, meteoric and tidal action (Sasekumar 1980, 1994; this study). Mudskippers are well adapted to rapid and drastic salinity changes (Evans et al 1999), even if long-term physiological responses to average salinity conditions had not been investigated. Therefore, high tide salinity isopleths (Robertson et al 1991; **Fig. 2**) and their biological meaning for mudskippers should be considered with caution.

No significant correlation was found between salinity and environmental aquaticity; nonetheless, if it is assumed that oxudercine gobies evolved from a marine most recent common ancestor, adaptation to freshwater may have been an important aspect of the eco-evolutionary radiation into semi-terrestrial habitats (Graham 1997). In fact, the striking increase of the number of species moving from Suki to the Fly delta is indeed a differential distribution along a salinity gradient, and seems to confirm the marine origin of these gobies. If the whole area is divided into three zones along this gradient (A-C: **Fig. 2**), only one species is found in A (*P. weberi*); two ones in B (*P. weberi* and *Z. confluentus*) and 10 species in C. This strongly suggests the presence of different degrees of euryhalinity among these mudskipper species.

Some alternative explanations are possible. At first, the vagility of the planktonic larvae of the species found only in C may not allow an efficient colonisation upriver. In fact, dense populations of *P. murdyi* and *P. weberi* were found in freshwater on the mud banks of the Adelaide river, Australia, NT, at about 50 km from the sea (G.P. pers. obs.). Therefore, at least in the case of *P. murdyi*, it is possible that strong currents and high suspended loads prevent larval dispersal up the Fly river.

Secondly, the lower Fly river may simply lack suitable habitats for the majority of these species. Only two species, *P. weberi* and *Z. confluentus*, were found in freshwater (A, B: **Fig. 2**): *Z. confluentus* is a relatively small fish which always remains in proximity of its burrow during low tide; while *P. weberi* is a highly mobile species which climbs and takes shelter among emerged roots and crevices in undercuts, or among buildups of logs and branches. All other species may be unable to find suitable habitats and conditions on the banks of the lower Fly river, swept by intense currents and wave action.

However, our observations show that: 1) the only species adapted to the most terrestrial conditions (*P. weberi*) is also found in freshwater; 2) at least for this species, salinity is *not* a factor affecting habitat distribution; and 3) *P. weberi* is found in the widest range of EA conditions, being highly eurytypic.

Complex and diverse mudskipper communities are found in coastal ecosystems characterised by wide tropical tidal mudflats (e.g. Milward 1974; Takita et al 1999). The broad range of gradually changing environmental conditions and the heterogeneity gradient formed by vegetation coverage offer a variety of potential ecological niches to mudskippers.

Tropical estuarine ecosystems, frequently characterised by strong tidal action, high river discharge, and high suspended loads, not only offer such conditions, but also wide salinity gradients upriver.

The scenario depicted by this study is typical of other tropical estuarine systems. In the Selangor estuary, *Pn. septemradiatus* was found in freshwater, like *P. weberi* (Khaironizam and Norma-Rashid 2003; G.P. pers. obs.); by the sea, in the same area, wide tidal mudflats host rich and diverse mudskipper communities (Takita et al 1999).

These findings and the present oxudercine phylogeny suggest that estuarine ecosystems were natural experimental laboratories where this lineage repeatedly gave origin to species with different degrees of adaptation to semi-terrestrial conditions and diverse amphibious lifestyles. This differentiation eventually led to species like *P. weberi*, that could radiate into freshwater habitats upriver, or at higher topographic levels.

While intra- and interspecific interactions seemingly exerted the selective pressures which promoted amphibious behaviours and terrestriality (Sayer and Davenport 1991; McNamara and Selden 1993; Sayer 2005), extreme euryhalinity seems one of the key preadaptations which allowed eurytypic mudskipper species to colonise more terrestrial habitats.

Mudskippers were proposed as convergent eco-evolutionary models to further our understanding of the environmental conditions and selective forces that drove the evolution of amphibious lifestyles in extinct semi-aquatic prototetrapods, about 385-

365 millions of years ago (Schultze 1999). In this respect, these findings seem to shift the focus on the age-old diatribe about the freshwater vs. seawater origin of terrestriality (e.g. Romer 1967; Graham 1997; Schultze 1999; Clack 2002). The sedimentological and geomorphic structure of Middle and Late Devonian estuarine and lower fluvial systems was being shaped by the explosive land colonisation of vascular plants, forming wide and gently sloping deposits of fine and organic sediments (Retallack 1997; Algeo et al 2001).

The ecology and evolution of mudskippers suggests that the first prototetrapods penetrated into terrestrial systems using estuaries and rivers as eco-evolutionary gateways from sea to land. Opportunities here seemingly knocked for any eurytypic aquatic sarcopterygian which could adapt to extreme salinity fluctuations and subaerial conditions.

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Captions

Figure 1

Cladogram of the Oxudercinae, rooted with the genus *Evorthodus* (Gobiidae: Gobionellinae), a hypothetical sister group of the Oxudercinae (Murdy, 1989). The genus *Oxuderces* is included in the tribe Oxudercini; all other genera here studied are included in the tribe Periophthalmini.

Figure 2

Study sites (1-17); salinity isopleths are drawn from Robertson *et al*, 1991 and this study. A, B, C: three river tracts with distinct mudskipper communities; tid.stat.: reference tidal station; upper panel: detail of the study sites on Purutu and other islands nearby; the creek flowing from Wapi village to the sea is also illustrated, while two lines indicate the position of the transects made along the Purutu channel (sites 16 and 17).

Figure 3

A-**S**: examples from photographed plots of the different environmental conditions. **A**: no vegetation coverage on a mud shoal in the middle of the lower Fly river (VC1; plot Lf03); **B**: grasses and bushes on the steep mud banks of the upper trait of Wapi Creek, Purutu I. (VC2, plot Pu01); **C**: pioneer mangrove forest (*Sonneratia lanceolata*) along the Purutu channel (VC3, plot Pu16); **D**: bottom of an ephemeral tidal inlet during low tide nearby Sturt I. (VC4, plot St04); **E**: nypah forest (*Nypa fruticans*), Purutu I. (VC5, plot Pu22); **F**: transition zone from the lowland rainforest to the freshwater swamp, Purutu I. (VC6, plot Pu23); **G**: mud banks of the mouth of a small creek: northern bank of the Fly, in front of Sturt I. (WB1, plot St02); **H**: larger tide pools on an exposed mudflat, Sisikura I. (WB2, plot Sk01); **I**: bottom of an empty inlet during low tide, nearby Sturt I. (WB3, same plot of D); **J**: drier area in a pioneer mangrove forest in front of Sturt I. (WB4, plot St07); **K**: absence of structural elements on the mud banks of a run-off channel nearby Tapila (SE1, plot Tp04); **L**: smaller vegetal debris on the floor of a deforested area colonised by grasses and shrubs of *Acanthus* sp.: mouth of the Purutu channel, Purutu I. (SE2, plot Pu08); **M**: pneumatophore zone (*S. lanceolata*) along the southern coasts of Wariura I. (SE3, plot Wa02); **N**: pneumatophores, logs and coarser vegetal debris in a *S. lanceolata* pioneer forest, Purutu I. (SE4, plot Pu17); **O**: heterogeneous vegetal debris on the mud banks of an irrigation channel in a sago palm plantation nearby Suki (SE5, plot Su01); **P**: trees, roots and heterogeneous vegetal debris inside a nypah forest (SE6, same plot of E); **Q**: undercut with exposed roots: erosive banks of the lower Fly (SE7, plot Lf01); **R**: litter, peat, trees, roots and heterogeneous vegetal debris on the floor of a freshwater swamp (SE8, same plot of F); **S**: buildup of floating logs accumulated in the upper tract of a creek nearby Sturt I. (SE9, plot St03). Photographs by G.P. and A.S.

Figure 4

A-J: mounted freshly dead specimens of the mudskippers of the Fly river and delta. Scale bars: 10 mm. **A**: *O. wirzi,* Purutu I. (plot Pu14); **B**: *Z. confluentus*, lower Fly river (Lf03); **C**: *S. histophorus*, female, Sisikura I. (Sk01); **D**: *B. caeruleomaculatus*, female, Sisikura I. (Sk01); **E**: *Pn. freycineti*, Purutu I. (Pu05); **F**: *P. novaeguineaensis*, Sisikura I. (Sk03); **G**: *P. murdyi*, female, Purutu I. (Pu05); **H**: *P. darwini*, Purutu I.

(Pu03); **I** and **J**: female and male of *P. weberi*, lower Fly river (St05 and Lf02, respectively). Photographs by G.P.

Figure 5

a: MCA of fish species/size classes (modalities) vs. environmental aquaticity (observations). species' names abbreviations use the first three letters of the scientific specific name; B.sp: *Boleophthalmus* sp.; P.sp: *Periophthalmus* sp.; two size classes are defined for each species: adults, and young/juveniles, indicated by the suffix "j". Young/juveniles of *Pn. freycineti* (fre), *P. darwini* (dar), and *P. novaeguineaensis* (nov) were not observed. Dashed ellipses outline four species groups (**W**, **IW**, **I**, and **T**), which respectively correspond to increasingly terrestrial conditions; and the group **V**, corresponding to a wide range of conditions. These groups correspond to the most supported clusters found by the AHC: node's similarity values are reported. Open squares: species; filled circles: environmental conditions; the four Cartesian quadrants are indicated in Roman numbers.

b: agglomerative hierarchical clustering (AHC) of species vs. salinity levels identifies three clusters (**FW**, **SI**, **SS**), respectively corresponding to increasing salinity levels. The cluster **SV** corresponds to a wide range of salinities.

Supplementary material

Appendix 1: examined museum specimens

7 type specimens were examined to confirm new records for New Guinea:

Periophthalmus darwini Larson and Takita, 2004: NTM-S-10554-004 (holotype: 45 mm SL; Micket Creek, Shoal Bay, Australia NT); NTM-S14400-006 (paratype: 38 mm SL female; beach south of Pichertaramoor, Melville I., Australia NT); *Periophthalmus murdyi* Larson and Takita, 2004: (5 paratypes: 36-39 mm SL, 4 females, 1 male; Bousteads Barramundi farm, Adelaide river, Australia, NT).

SL: standard length. AMS: Australian Museum of Sydney, Sydney, Australia; NTM: Museum and Art Gallery of the Northern Territory, Darwin, Australia.

Appendix 2: reference collection

*Boleophthalmus caeruleomaculat*us McCulloch and Waite, 1918: **2** specimens from 1 locality, Western Province, PAPUA NEW GUINEA: Fly river delta, Sisikura I.; XXX (xxx mm SL, female), XXX, (xxx mm SL, male), exposed mudflat, in front of a *Sonneratia alba* pneumatophore zone, inside burrows. 24 September 2007. *Oxuderces wirzi* (Koumans, 1938): **2** specimens from 1 locality, Western Province, PAPUA NEW GUINEA: Fly river delta, Purutu I.; XXX (xxx mm SL), exposed mudflat, 28-29 September 2007; XXX, (xxx mm SL), exposed mudflat, nearby the mouth of a creek, 29 September 2007. *Periophthalmodon freycineti* (Quoy and Gaimard, 1824): **2** specimens from 1 locality, Western Province, PAPUA NEW GUINEA: Fly river delta, Purutu I., Wapi creek; XXX, (xxx, xxx mm SL), exposed mud banks nearby the water edge, 24 September 2007. *Periophthalmus darwini* Larson and Takita, 2004: **4** specimens from 1 locality, Western Province, PAPUA NEW GUINEA: Fly river delta, Purutu I., Wapi creek; XXX, (xxx-xxx mm SL), exposed and vegetated mud banks, 24

September 2007. *Periophthalmus murdyi* Larson and Takita, 2004: **4** specimens from 2 localities, Western Province, PAPUA NEW GUINEA, Fly river delta, Purutu I.: Wapi Creek and Purutu channel; size range xxx-xxx mm SL: XXX, 1 (xxx mm SL), exposed mud banks nearby the water edge, Wapi Creek, *ibid.*, 24 September 2007; XXX, 3 (xxx-xxx mm SL), seaward margin of a nypah forest, at the transition with a *Sonneratia lanceolata* pioneer forest, Purutu channel, *ibid.*, 29 September 2007. *Periophthalmus novaeguineaensis* Eggert, 1935: **2** specimens from 2 localities, Western Province, PAPUA NEW GUINEA, Fly river delta: Sisikura I. and Purutu channel; XXX, (xxx mm SL), exposed mudflat, in front of a *Sonneratia alba* pneumatophore zone, Sisikura I., *ibid.*, 24 September 2007; XXX, (xxx mm SL), *Sonneratia lanceolata* pioneer mangrove forest and grasses (Cyperaceae), Purutu channel, *ibid.*, 29 September 2007. *Periophthalmus weberi* Eggert, 1935: **5** specimens from 3 localities, Western Province, PAPUA NEW GUINEA, lower Fly river and delta: Suki, Sturt I., and Purutu channel; size range xxx-xxx mm SL: XXX (xxx mm SL, male), mud banks of an irrigation channel of a sago plantation (*Metroxylon sagu*), Suki, *ibid.*, 18 September, 2007; XXX, **2** (xxx mm SL, male; xxx mm SL, female), exposed mud banks of a small creek with vegetal debris, Sturt I., *ibid.*, 19 September, 2007; XXX **2** (xxx mm SL, male; xxx mm SL, female), nypah forest, more humid areas, Purutu channel, *ibid.*, 29 September, 2007. *Scartelaos histophorus* (Valenciénnes, 1837): **4** specimens from 2 localities, Western Province, PAPUA NEW GUINEA, Fly river delta: Sisikura I. and Purutu channel; size range xxx-xxx mm SL: XXX **2** (xxx mm SL, male; xxx mm SL, female), exposed mudflat, in front of a *Sonneratia alba* pneumatophore zone, Sisikura I., *ibid.*, 24 September, 2007; XXX **2** (xxx mm SL, male; xxx mm SL, female), exposed mudflat and *Sonneratia lanceolata* pioneer forest, Purutu channel, *ibid.*, 29 September, 2007.

Zappa confluentus (Roberts, 1978): **4** specimens from 1 locality, Western Province, PAPUA NEW GUINEA, lower Fly river: mainland in front of Sturt I.; size range xxx-xxx mm SL: XXX, mud banks with few trees of *Sonneratia lanceolata*, nearby tide pools, 17 September, 2007.

Appendix 3: field identification and ethological notes

All the described species are consistent with the most updated taxonomic keys (Murdy, 1989; Larson and Takita, 2004). Nonetheless, no data on field identification of mudskippers of Papua New Guinea were found in literature.

O. wirzi is an extremely inconspicuous species, TL< 10 cm (Murdy, 1989); it was found in very fine sediments and semiliquid muds on mudflats and mouths of run-off channels. It can be spotted when surfacing with the eyes, crawling on the mud covered by very shallow water. The banded dark dorsal pattern is the only trait visible in the field (**Fig. 4A**). No sexual dimorphism was observed.

Z. confluentus is locally abundant: several individuals per square meter were found on mudflats and exposed mud banks during low tide, when they continuously perform terrestrial activities; they shuttle back and forth from their small burrows to the water edge, feeding with the mouth pressed against the substrate, and skimming the mud surface by side to side head movements. Roberts (1978) reported that this species is not able to maintain an upright position on the pelvic fins. According to our observations, the locomotory behaviours of adults are comparable to those of *Boleophthalmus* spp. It is possible that Roberts observed the escaping response of shoals of juveniles, whose jumping behaviour is less precise than in adults. During flood tide *Z. confluentus* enters it burrow. It reaches 6-7 cm TL (Allen, 1991; this study), presents a greyish inconspicous colouration (**Fig. 4B**), and an eely shape. No sexual dimorphism was observed.

S. histophorus is also greyish and slender in shape, but its head is proportionally longer and eyes more dorsally positioned than in *Z. confluentus*. Adults are also larger than the former species (up to approx. 15 cm TL: Rainboth, 1996), and both sexes present a very typical thin, pointed, and well visible first dorsal fin, which is intermittedly erected while feeding or during intraspecific interactions. This species was found on open mudflats, pneumatophore zones, and even inside pioneer mangrove forests, but always nearby tide pools. Mature males are larger than females, and perform typical ‘tail stands’ to attract them (Townsend and Tibbetts, 2005), being easily spotted from several meters in the distance on open areas. Townsend and Tibbetts (2005) reported that males have eyes with “white borders”. By observations from this study and of Australian specimens (G.P., *unpubl. obs.*), we would describe this dimorphism as the presence of a more intense pigmentation of females’ orbits. Nonetheless, this trait is not easily observed in the field, especially when irises are dilated.

B. caeruleomaculatus is the second largest species among the observed mudskippers (up to approx. 20 cm TL: Murdy, 1989): adults are found on open mudflats, and are easily spotted from the distance. When approached at a distance of approx. less than 10 m, they immediately took refuge into their deep burrows. When undisturbed, these fishes perform intense territorial activities with prolonged fin displays. In the field they are easily identified by the bright blue colouration of the inner side of the eye’s dermal cup (Murdy, 1989; **Fig. 4D**), while the dense and bright blue speckles on flanks, head and large dorsal fins are not always visible in the field. In adults, no dark diagonal bars are present on flanks. Adult females (captured specimens and museum material) presented elongated first dorsal fin spines, while males have no elongated spines.

Boleophthalmus sp. was found on creek mud banks and in pioneer mangrove forests, while smaller individuals were also found in wet areas inside nypah forests. This species is currently under description.

Pn. freycineti is one of the most easily observed species, rapidly surfing and swimming with short bursts along the water edge of tidal creeks and open mudflats. They are the largest known mudskippers (TL> 28 cm: examined museum material); adults dig large burrows on mud creek banks and on mudflats, with typical funnel shaped openings, 20-60 cm in diameter at ground level. In the field, its most conspicuous trait is the dorsal horizontal black stripe coursing posteriorly from the orbits to the dorsal attachment of the opercle, up to the caudal peduncle (Murdy, 1989). When illumination is scarce, a dorsal banded dark pattern is visible, which interrupts the dark stripe; nonetheless, the anteriormost portion of the horizontal stripe is always visible (**Fig. 4E**). Other useful traits are the numerous whitish speckles scattered on cheeks, opercles and flanks; and two parallel and prominent horizontal protuberances on the snout, visible also in smaller specimens (**Fig. 4E**). The first dorsal fin is relatively small and inconspicuous in both sexes (even if always proportionally larger than in females of *P. weberi*), while the second dorsal fin is relatively tall, reaching the dorsal attachment of the caudal fin when appressed. No sexual dimorphism was observed.

Upon close inspection, pelvic fins of all the previous species are completely fused into a round disk.

P. darwini is one of the smallest oxudercine gobies, reaching about 5 cm TL (Larson and Takita, 2004), and is the only known species in which both males and females have atrophic first dorsal fins; this trait is visible in the field also when fins are appressed (**Fig. 4H**). For this reason, they could be confused with young females of *P. weberi* (**Fig. 4I**); nonetheless, they never present neither horizontal stripes behind eyes, nor bright blue speckles on head and flanks, nor a snout crease. The dark dorsal, diagonal saddle-like blotches are frequently visible. During low tide, they were found along the mud banks of creeks, and on the bottom of ephemeral inlets in forested areas. No sexual dimorphism was observed.

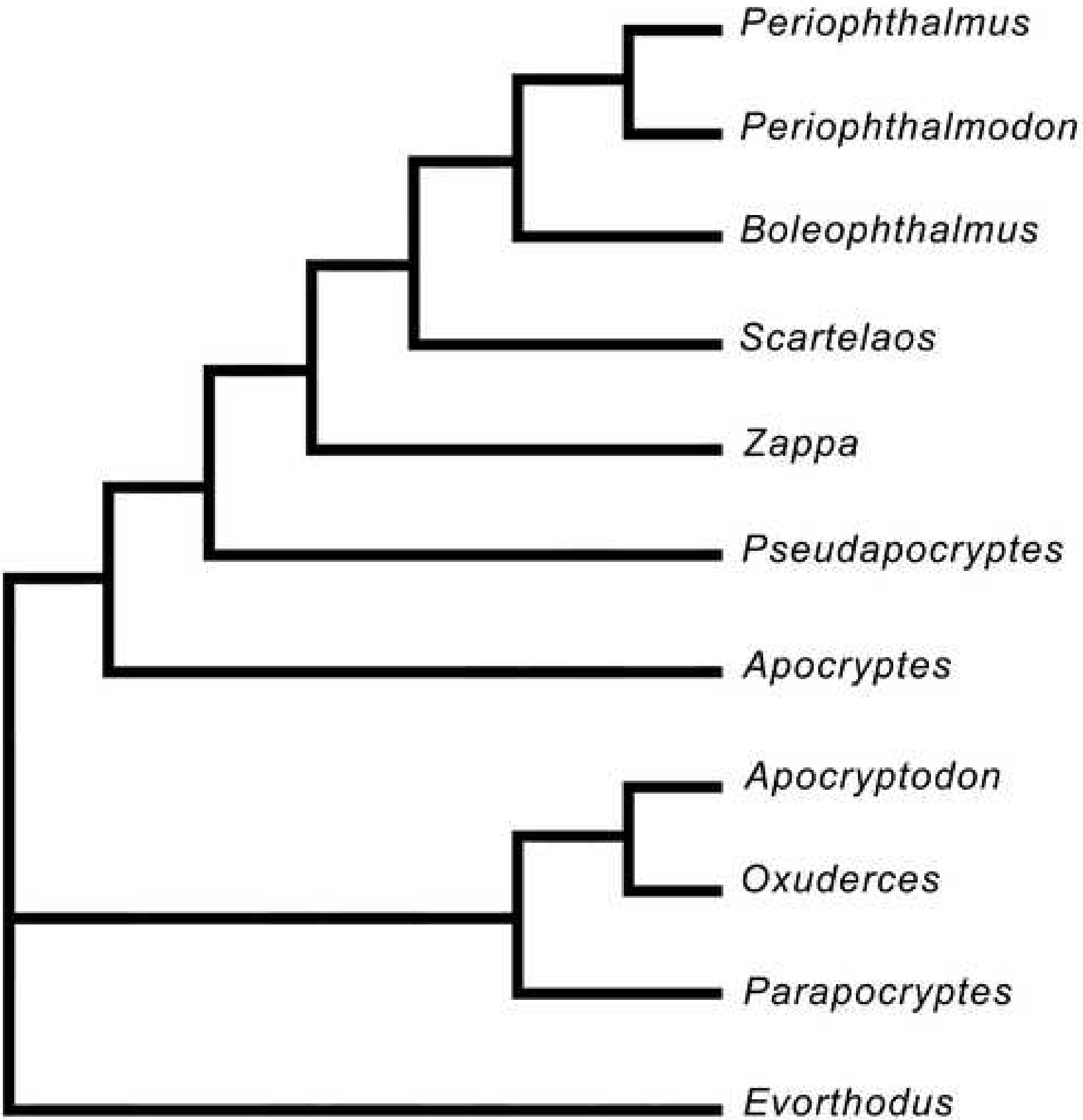
P. murdyi reaches about 6 cm TL (Larson and Takita, 2004); it can be abundant in relatively open areas, frequently nearby the water edge of small pools, where it feeds. The most useful diagnostic trait in the field is the presence of numerous and small sky blue spots on snout, cheeks, opercles, and flanks (**Fig. 4G**). Reproductive males are darker than females in background colouration and may presents brighter and larger sky blue spots.

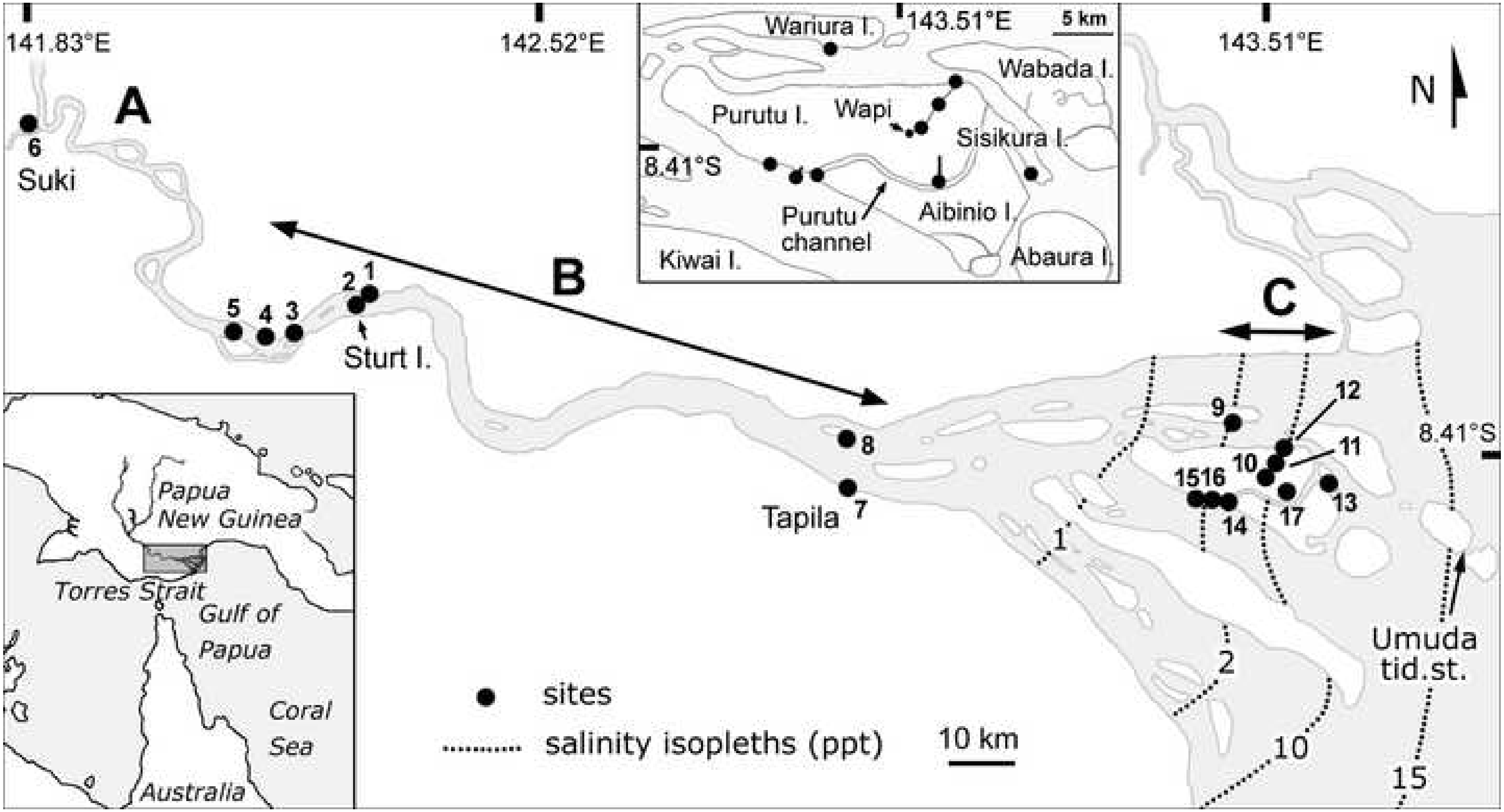
P. novaeguineaensis is a stocky and moderately large species of *Periophthalmus*, reaching 9 cm TL (Larson and Takita, 2004). It was found in level and open areas with soft and wet mud, within few meters from the pioneer vegetation, and in pioneer mangrove forests. Its most prominent trait is the presence of reddish spots on flanks and cheeks (**Fig. 4F**), well visible in the field. No sexual dimorphism was observed.

Periophthalmus sp. was always found in vegetated areas, inside pioneer mangrove and nypah forests. Also this species is currently under description.

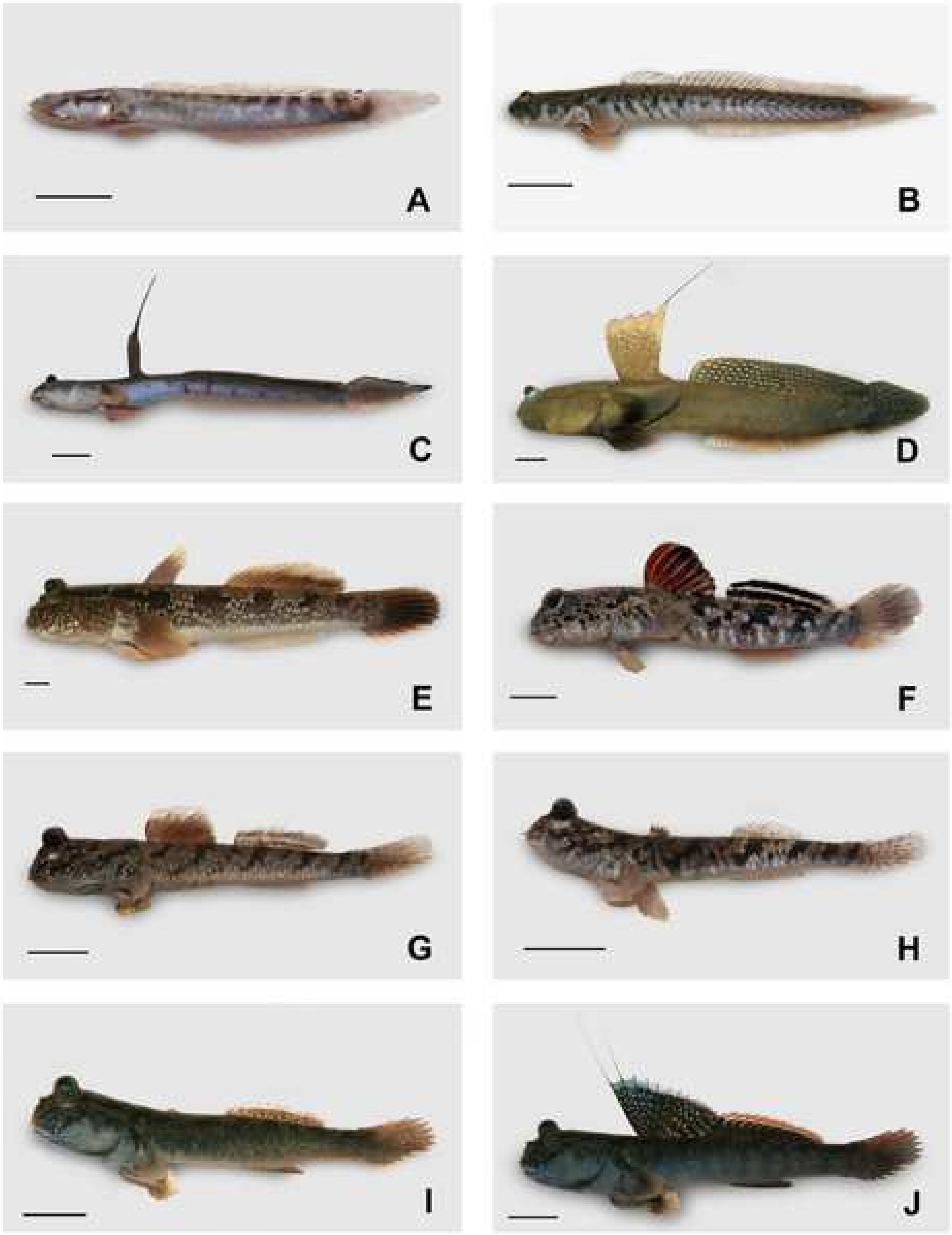
Finally, *P. weberi* presents a marked sexual dimorphism: reproductive males have an intense and variable coloration, and can rapidly turn into a deep blue background colour (**Fig. 4J**). Adult males also have a very large first dorsal fin, posteriorly contiguous to the second dorsal fin, and with the two first spines much elongated (**Fig. 4J**), visible also when the fin is appressed. Females have atrophic, barely visible first dorsal fins (**Fig. 4I**), and are yellowish to brownish in background colour. A dorsal dark banding pattern may be displayed, especially in the young. The most visible diagnostic traits in the field are an irregular though prominent horizontal dark stripe behind the eyes, reaching posteriorly to the dorsal attachment of the operculum (**Fig. 4I**), that may reach the caudal peduncle; and a conspicuous horizontal crease on the snout. These traits may recall *Pn. freycineti*, but *P. weberi* lacks the conspicuous white speckles on head and flanks found in the former species. Instead, bright blue iridescent speckles may be visible on flanks and cheecks, and scattered black speckles along flanks. Dorsal fins have a unique and intense colouration, especially in males (**Fig. 4J**), but are not frequently observed in the field. Burrows of this species were observed on the soft mud banks of creeks and ephemeral inlets: single main openings may have slightly raised rims, with evident mud gobbets scattered around them. *P. weberi* is one of the few mudskippers which escapes towards land when chased along the water edge, taking shelter among the vegetation, or among roots in undercuts, and inside sesarmid crab burrows.

Upon close inspection, all *Periophthalmus* spp. present species-specific pelvic fins morphologies (Larson and Takita, 2004).


Periophthalmus
Periophthalmodon
Boleophthalmus
Scartelaos
Zappa
Pseudapocryptes
Apocryptes
Apocryptodon
Oxuderces
Parapocryptes
Evorthodus


141.83°E
142.52°E
143.51°E
143.51°E
5 km
A
B
C
N
Wariura I.
Wabada I.
Purutu I.
Wapi
Sisikura I.
8.41°S
Purutu channel
Aibinio I.
Kiwai I.
Abaura I.
6
Suki
5
4
3
2
1
Sturt I.
8
Tapila
7
9
12
11
10
1516
14
17
13
8.41°S
1
2
10
15
Umuda tid.st.
Papua New Guinea
Torres Strait
Gulf of Papua
Coral Sea
Australia
● sites
salinity isopleths (ppt)
10 km

colour figure


A
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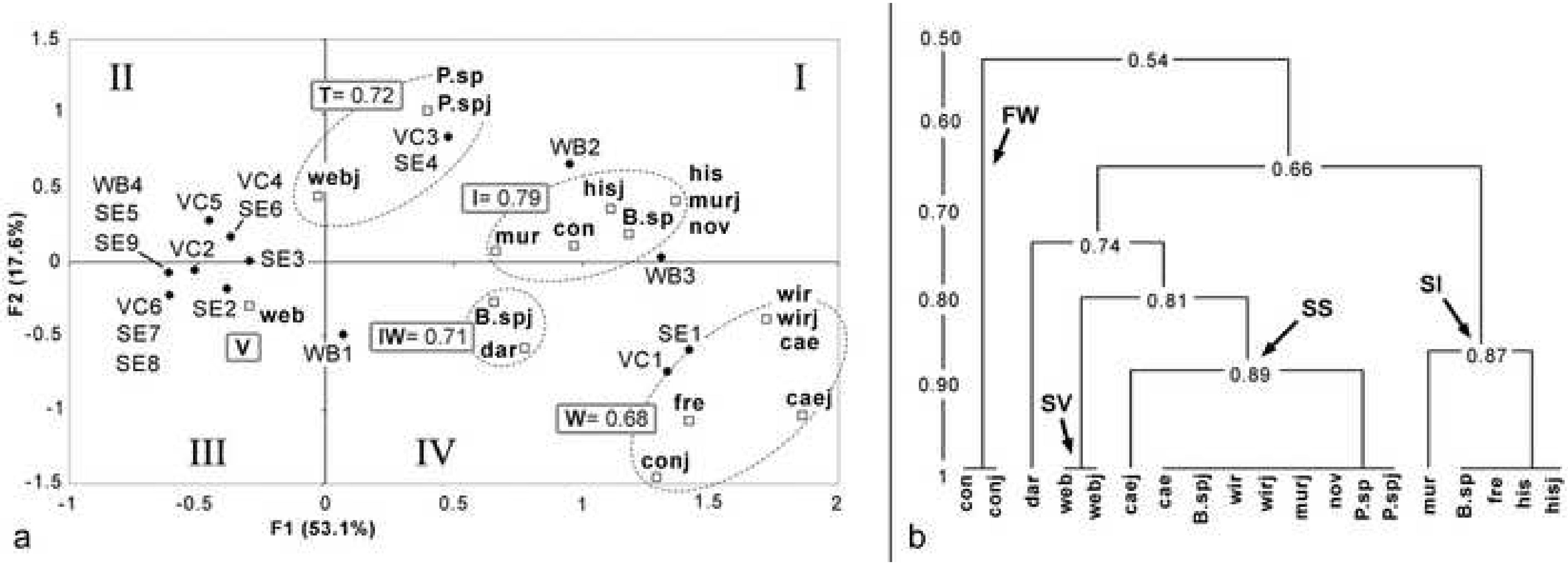
1.5
1
0.5
0
-0.5
-1
-1.5
-1
-0.5
0
0.5
1
1.5
2
F2 (17.6%)
F1 (53.1%)
II
I
III
IV
T= 0.72
P.sp
P.spj
VC3
SE4
webj
WB2
I= 0.79
hisj
his
murj
nov
B.sp
con
mur
WB3
WB4
SE5
SE9
VC4
SE6
VC5
VC2
SE3
VC6
SE7
SE8
SE2
web
V
WB1
IW= 0.71
B.spj
dar
SE1
VC1
wir
wirj
cae
fre
caej
W= 0.68
conj
a
0.50
0.60
0.70
0.80
0.90
1
0.54
FW
0.66
0.74
0.81
SS
SI
0.87
0.89
SV
con
conj
dar
web
webj
caej
cae
B.spj
wir
wirj
murj
nov
P.sp
P.spj
mur
B.sp
fre
his
hisj
b

Table 1. Species/size classes distribution and environmental parameters

sites	plots	[a]TC	con	conj	cae	caej	B.sp	B.spj	wir	wirj	fre	dar	mur	murj	nov	web	webj	P.sp	P.spj	his	hisj	[b]SS	[c]SE	[d]WB	[e]VC
S1	St01	X	1																			1	4	2	3
	St02	X	1																			1	2	1	1
	St03	X														1						1	9	1	1
	St04	X														1	1					1	9	3	4
	St06	X	1	1																		1	1	1	1
	St07	X															1					1	4	4	3
S2	St05	X														1	1					1	5	1	2
S3	Lf01	Y/P														1	1					1	7	1	1
S4	Lf02	Y/P														1	1					1	9	3	2
S5	Lf03	Y/P	1																			1	1	4	1
S6	Su01	X														1						1	5	1	1
S7	Tp01	Y/P	1																			1	3	3	1
	Tp02	Y/P	1														1					1	1	1	1
	Tp03	Y/P														1						1	5	1	2
	Tp08	Y/A	1																			1	1	3	1
S8	Tp04	X	1																			1	1	1	1
	Tp05	X	1																			1	1	1	1
	Tp06	X	1																			1	1	1	1
	Tp07	Y/A														1						1	7	4	2
S9	Wa01	X					1				1									1		2	1	3	1
	Wa02	X											1								1	2	3	3	1

[a] tidal conditions; [b] salinity (SS1-4); [c] structural elements (SE1-9); [d] water bodies (WB1-4); [e] vegetation coverage (VC1-6). Other abbreviations as in **Fig. 5.**

Table 1. Species/size classes distribution and environmental parameters (continued)

sites	plots	TC	con	conj	cae	caej	Bsp	Bspj	wir	wirj	fre	dar	mur	murj	nov	web	webj	Psp	Pspj	his	hisj	SS	SE	WB	VC
S10	Pu01	Y/P										**1**				**1**						3	1	1	2
	Pu02	Y/P										**1**				**1**						3	2	4	2
	Pu03	Y/P										**1**						**1**				3	2	1	2
	Pu04	Y/P										**1**										3	6	1	4
S11	Pu05	X					**1**				**1**	**1**	**1**									3	1	1	1
	Pu06	X										**1**										3	6	3	4
S12	Pu07	X					**1**				**1**	**1**										3	1	1	1
S13	Sk01	X			**1**															**1**		4	1	2	1
	Sk02	X					**1**				**1**											4	1	1	1
	Sk03	X													**1**					**1**		4	1	2	1
S14	Pu08	X											**1**									3	2	3	2
	Pu09	X														**1**						3	2	4	2
	Pu10	X																		**1**		3	1	3	1
	Pu11	X			**1**		**1**			**1**										**1**		3	1	3	1
	Pu12	X									**1**											3	1	1	1
S15	Pu13	X														**1**						3	6	3	5
	Pu14	X							**1**											**1**		3	1	2	1
S16	Pu15	Y/A				**1**		**1**	**1**	**1**				**1**						**1**	**1**	4	1	3	1
	Pu16	Y/A					**1**						**1**		**1**					**1**	**1**	3	4	3	3
	Pu17	Y/A											**1**	**1**				**1**		**1**	**1**	3	4	3	3
	Pu18	Y/A											**1**	**1**			**1**	**1**	**1**	**1**	**1**	3	4	2	3
	Pu19	Y/A						**1**								**1**	**1**	**1**	**1**			4	6	3	5
	Pu20	Y/A						**1**								**1**	**1**	**1**	**1**			3	6	3	4
S17	Pu21	Y/P											**1**									4	2	1	2
	Pu22	Y/A														**1**		**1**				3	6	3	5
	Pu23	X														**1**						1	8	3	6

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Cyb499

First record of *Periophthalmus walailakae* (Gobiidae: Oxudercinae) from Peninsular Malaysia

by

Gianluca POLGAR (1) & Md. Zain KHAIRONIZAM (2)

RÉSUMÉ. - Premier signalement de *Periophthalmus walailakae* (Gobiidae: Oxudercinae) en Malaisie péninsulaire.

Periophthalmus walailakae Darumas & Tantichodok est signalé pour la première fois en cinq endroits le long des côtes occidentales de la Malaisie péninsulaire (Selangor, Johor) ; 22 spécimens collectés ont été comparés au matériel type. Ces résultats prolongent la distribution documentée de ces espèces à la grande partie de la côte occidentale de la péninsule de Malacca. Des notes morphologiques et écologiques et la présence des espèces associées et sympatriques sont également fournies.

Key words. - Gobiidae - Oxudercinae - *Periophthalmus walailakae* - ISW - Peninsular Malaysia - Sympatric species - First record.

Figure 1. - Live (**A**) and preserved (**B**) specimen of *Periophthalmus walailakae*. **A**: Male (109.5 mm SL, MSNG 51393); **B**: Female (107.5 mm SL, KMZ-NYR000722S (8)). Scale bar: 10 mm. Photographs by G. Polgar. *[Spécimen vivant (**A**) et préservé (**B**) de* P. walailakae. ***A** : Mâle ; **B** : Femelle. Échelle : 10 mm.]*

Mudskippers (Gobiidae: Oxudercinae; Murdy, 1989) are a conspicuous component of tropical intertidal ecosystems. These fishes are abundant on tropical mudflats and mangrove forests from Western Africa in the Atlantic, to the entire Indo-Pacific region.

Periophthalmus Bloch & Schneider, 1801, with 17 species, is the richest genus (Murdy, 1989; Lee *et al*., 1995; Murdy and Takita, 1999; Larson and Takita, 2004).

P. walailakae Darumas & Tantichodok, 2002 was described from the province of Ranong, Thailand. Jafaar *et al*. (2006) reported its presence in Singapore. Khaironizam and Norma-Rashid (2002) reported unidentified *Periophthalmus* specimens from Selangor, Malaysia. These specimens are here discriminated as *P. walailakae* and morphologically compared with other Malaysian specimens from Selangor and Johor, extending the actual documented distribution of this species in certain localities along the west coast of the Malacca Peninsula, from Thailand to Singapore (Fig. 2).

MATERIAL AND METHODS

Fishes were caught at low tide by hand nets. The live colour pattern was recorded in the field and in laboratory. Twenty-two fish were fixed in 10% formalin for morphological analyses, while three specimens (MSNG 54140 (2) and MSNG 54141 (1)) were fixed and preserved in 95% not denatured ethanol for future molecular analyses. A binocular microscope (15-60X) and a dial calliper were used to collect meristic and morphometric data. The number of teeth rows on the upper jaw was also examined (Murdy, 1989). Methods for counts and morphometrics follow Hubbs and Lagler (2004) and Murdy (1989) except for pectoral-fin length, which is the straight-line distance from the dorsal attachment of the muscular pectoral-fin base to the tip of the fin rays. Two paratypes were also examined for comparison (PMBC 19550 and PMBC 19551). Specimens were deposited in the Zoological Museum of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur (KMZ-NYR); and in the Museo Civico di Storia Naturale “Giacomo Doria”, Genova, Italy (MSNG).

PERIOPHTHALMUS WALAILAKAE DARUMAS & TANTICHODOK, 2002

Material examined

One female, (paratype) (PMBC 19550), Thailand: Ao Phang-nga, Phang-nga; one male, (paratype) (PMBC 19551), ibid.; one male, (109.5 mm SL) (MSNG 51393), Malaysia: Selangor, Kuala Selangor, coll. G. Polgar, 8 Aug. 1996; 16 ex., (54.3-85.9 mm SL) (KMZ-NYR-M UMKL 5015), Malaysia: Selangor, Morib, coll. M.Z. Khaironizam, 12 Jan-14 Sep. 1999; 4 ex., (91.6-113.4 mm SL) (KMZ-NYR-S UMKL 5015), Malaysia: Selangor, Sementa, coll. M.Z. Khaironizam, 16 May-22 Jul. 2000; one ex., (74.1 mm SL) (KMZ-NYR-M UMKL 5015), Malaysia: Selangor, Morib, coll. M.Z. Khaironizam, same date as above; two ex., (41, 39 mm SL) (MSNG 54140), Malaysia: Johor, Pulau [=Island] Kukup, coll. G. Polgar, 12 Nov. 2006; one ex., (68 mm SL) (MSNG 54141), Malaysia: Johor, Tanjung [=Cape] Piai, coll. G. Polgar, 21 Oct. 2006.

Diagnosis

A species of *Periophthalmus* with the innermost pelvic fin rays joined by a basal membrane for their entire length: pelvic fins form a round disk. Strong pelvic frenum present. Series of dark brown speckles on caudal and pectoral-fin rays are present in live and preserved specimens. No sexual dimorphism of the dorsal fins. Length of anal-fin base % SL 15.0-18.4 (mean 17.0); length of second dorsal-fin base % SL 17.0-22.5, mean 19.0; head depth % SL 19.5-22.9, mean 21.1 (Tab. I).

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Cybium proofs: 3 p.

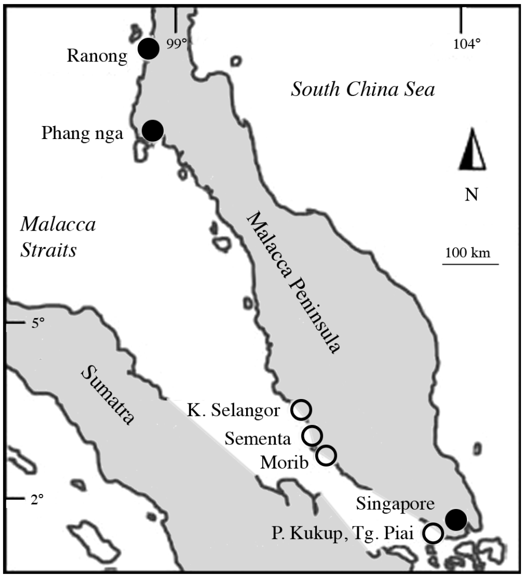

Figure 2. - Recorded distribution of *Periophthalmus walailakae*. ○: old records; ●: new record (this study). *[Distribution enregistrée des* P. walailakae. ○ *: signalements anciens ;* ● *: nouveau signalement (présente étude).]*

Coloration pattern

Based on a photo of a live specimen (MSNG 51393: 109.5 mm SL, male: Fig. 1A), and on observations of other live and freshly dead specimens: background coloration brownish, with numerous, irregular white to pale yellow speckles on flanks, cheeks and throat, as large as or larger than the exposed field of the underlying scale. 4-7 diagonal, saddle-like, dorsal and irregular dark bars; irregular black blotches on cheeks. Belly white. First dorsal fin proximally dark brown and black for the distal third; margin white. Second dorsal fin with transparent background, medial dark brown stripe and reddish distal margin; 2-3 horizontal series of dark speckles on rays in some specimens. Anal fin white. Pectoral and caudal fin background greyish, with series of dark brown spots along rays. Pelvic fins ventrally white; dorsally rays pale brown.

In preservation (Fig. 1B), body coloration as in live specimens, but with less defined darker or paler speckles and blotches. Belly yellowish-grey. Fins as in live specimens, but with transparent margins. Anal and pelvic fins yellowish-grey. Ventral peritoneum dark brown.

Remarks

The diagnostic dorsal and anal fin counts (Darumas and Tantichodok, 2002; Larson and Takita, 2004) were not confirmed by this analysis and by inspection of paratypes (PMBC 19550 and PMBC 19551), which had counts of I, 11 for both the anal and D2 fins, within the range of other congeneric species.

P. walailakae, *P. spilotus* Murdy and Takita, 1999 and *P. chrysospilos* Bleeker, 1852 are the only three *Periophthalmus* species with totally fused pelvic fins.

P. walailakae is the only one with series of dark speckles on caudal and pectoral fin rays. Few other characters show some differences (Tab. I; Murdy, 1989; Murdy and Takita, 1999).

In *P. spilotus*, length of anal-fin base % SL: range 21.8-29.0 (mean 24.2); length of second dorsal-fin base % SL: 22.6-26.7 (24.4); and number of elements of the second dorsal fin: 14-15 (14.1); in *P. chrysospilos*, head depth % SL: 14.6-20.6 (17.6).

Table I. - Ranges, means and standard deviations (sd) of selected morphometric and meristic data for the 22 *Periophthalmus walailakae* examined in this study. *[Moyennes, intervalles et écarts types (sd) des données morphométriques et méristiques choisies pour les 22* P. walailakae *examinés dans cette étude.]*

	Mean	Range	sd
Standard length (mm)	65.8	45.8-109.5	18.6
Morphometrics (% of standard length)			
Body depth	15.8	14.6-17.3	0.8
Head length	29.3	27.7-30.3	0.7
Head depth	21.1	19.5-22.9	0.9
Length of first dorsal-fin base	13.8	11.0-18.3	1.8
Length of second dorsal-fin base	19.0	17.0-22.5	1.1
Length of anal-fin base	17.0	15.0-18.4	0.9
Length of pectoral-fin base	24.1	22.5-26.1	1.0
Length of pelvic fins	13.4	12.6-14.9	0.5
Least depth of caudal peduncle	9.4	8.9-10.5	0.4
Length of caudal fin	25.1	21.7-28.2	2.0
Meristic counts			
Pectoral-fin rays	15.0	15-16	0.2
Longitudinal scale count	68.3	66-72	1.7
First dorsal-fin elements	8.0	7-9	0.8
Second dorsal-fin total elements	12.0	12-13	0.2
Anal-fin total elements	12.0	11-13	0.3
Transverse scale counts (TRDB)	16.7	15-20	1.3

Distribution and ecological notes

All observations were made at low tide (Fig. 2). In Sementa (3°5'N; 101°21'E) and Kuala Selangor (3°21'N; 101°15'E), *P. walailakae* was found in mixed mangrove forests (high shore, Sasekumar, 1980): along small inlets, sympatric with subadults and juveniles of *Boleophthalmus boddarti* (Pallas, 1770), and *Periophthalmodon schlosseri* (Pallas, 1770); and in drier areas, up onto the landward fringe, in association with *P. novemradiatus* (Hamilton, 1822), *P. spilotus*, and *P. gracilis* Eggert, 1935. In the small mangrove formations of Morib (2°45'N; 101°26'E) it was found nearby tide pools, at the transition from the low to the high shore, together with *P. argentilineatus* Valenciennes, 1837 and *P. gracilis*. In Tanjung Piai (1°15'N; 103°30'E) few *P. walailakae* subadults were found on the seaward side of a high debris step that separates low and high shore, preventing the tide to enter the high forest. Sympatric species in these atypical conditions were adult and young *P. argentilineatus*, *P. chrysospilos*, and *Periophthalmodon schlosseri*; young *Boleophthalmus dussumieri* Valenciennes, 1837, and young *B. boddarti*; also few specimens of *P. gracilis* and *P. novemradiatus* were present. In Pulau Kukup (1°20'N; 103°25'E) young specimens were found in the low shore, in association with young *P. chrysospilos*.

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