

## Amphidromy and marine larval phase of ancestral gobioids *Rhyacichthys guilberti* and *Protogobius attiti* (Teleostei: Rhyacichthyidae)

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**Abstract.** Even if amphidromous fish species contribute most to the diversity of fish communities in the tropical insular rivers, their biological cycle remain poorly known. For the first time, the otolith elemental composition and microstructure of two ancestral gobioids, *Rhyacichthys guilberti* and *Protogobius attiti*, were investigated to describe their biological cycle and pelagic larval duration (PLD). The otolith analysis using a femtosecond laser ablation coupled to an inductively coupled plasma–mass spectrometer (fs-LA-ICP-MS) revealed an amphidromous life history for *R. guilberti* and it suggested a progressive habitat shift from a marine habitat to a freshwater environment for *P. attiti*. For the first time, an endemic species, *P. attiti*, showed longer and more variable PLD ( $55.2 \pm 13.5$  days) than did a widespread one (*R. guilberti*: ~30 days). These results need to be confirmed by analysing more samples but suggest that factors other than the PLD control endemism and dispersal processes. In association with this first description of the biological cycle for both species, such an approach is a prerequisite for the management and conservation of both patrimonial species.

**Additional keywords:** diadromy, freshwater gobies, otolith microchemistry, tropical islands.

Received 11 June 2013, accepted 21 November 2013, published online 16 June 2014

### Introduction

The tropical insular river systems in the Indo-Pacific are mainly colonised by diadromous fish species (Keith 2003) and, more specifically, by amphidromous fish (McDowall 2007). The adults grow, feed and reproduce in rivers. The newly hatched larvae drift downstream towards the sea where they spend 2–6 months (Lord *et al.* 2010; Taillebois *et al.* 2012). Then post-larvae come back to rivers, recruit in estuaries and generally undergo a metamorphosis (Iida *et al.* 2008; Keith *et al.* 2008; Taillebois *et al.* 2011) while migrating upstream to settle. The oceanic dispersal of these freshwater organisms represents an essential element in the persistence and structuring of populations at both the local and regional scales (Keith *et al.* 2011). Nevertheless, the processes that occur during the pelagic larval phase remain poorly known (Radtke and Kinzie 1996; Shen *et al.* 1998; Taillebois 2012). As the amphidromous species contribute most to the diversity of fish communities in the tropical Indo-Pacific insular river systems, it is essential to

understand these processes to implement conservation measures (Keith and Lord 2011).

Among the Indo-Pacific insular fish community, the Rhyacichthyidae family includes two genera (*Protogobius* Watson & Pöllabauer, 1998, and *Rhyacichthys* Boulenger, 1901) and three species (*Rhyacichthys aspro* Valenciennes, 1837; *Rhyacichthys guilberti* Dingerkus & Séret, 1992; *Protogobius attiti* Watson & Pöllabauer, 1998). *R. guilberti* is known from the Northern Province of New Caledonia and from Vanuatu to Papua (Keith 2002). In Vanuatu, Papua and Solomon Islands, adults are targeted as a food resource (Keith *et al.* 2010). *P. attiti* is a rare and endemic fish of southern New Caledonia, considered as endangered by the IUCN (Larson 2012). In addition to conservation interests, Rhyacichthyidae are of particular interest in regard to the evolutionary history of Gobioidae because they are sister group of all Gobioidae (Hoese and Gill 1993; Keith *et al.* 2011).

Otoliths are well known paired, calcified structures in the fish inner ear (Campana 1999). They have long been used for age

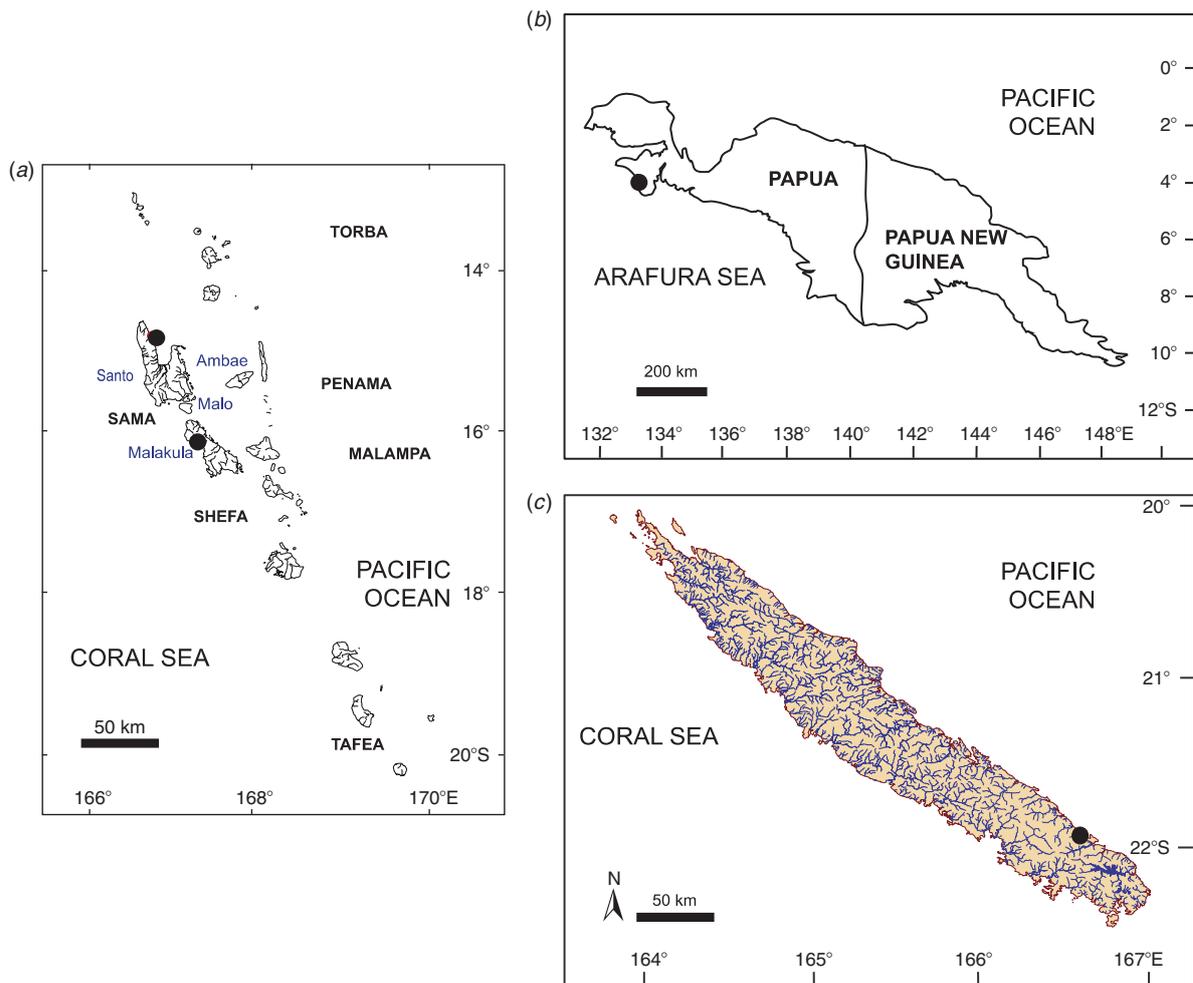


Fig. 1. Sampling sites (black dots) in (a) Vanuatu, (b) Papua and (c) New Caledonia.

estimation in many fish species, especially in diadromous species (Lecomte-Finiger 1999; Radtke *et al.* 2001; Shen and Tzeng 2008). Several studies on Gobiidae have revealed a check-mark deposition at the time of post-larval recruitment linked to a decrease in the calcareous growth rate (Shen and Tzeng 2002; Keith *et al.* 2008). The number of increments between the core and the metamorphosis check mark is therefore an estimation of their pelagic larval duration (PLD) (Yamasaki *et al.* 2007; Feutry *et al.* 2012). The elemental composition of the otolith is used to reconstruct the migratory environmental history (Daverat and Tomas 2006; Shiao *et al.* 2006). Among the elements trapped in the otolith daily increments, strontium (Sr) is commonly used to reconstruct diadromous fish migratory movements (Arai and Hirata 2006; Feutry *et al.* 2011). Sr content in the otolith of teleost fish is positively correlated with ambient salinity (Tzeng 1996; Secor and Rooker 2000; Tabouret *et al.* 2010). Elevated Sr : calcium (Ca) ratios in fish otoliths are widely accepted as proof of occupation of marine habitats (Kalish 1990; Shen *et al.* 1998). Recent findings have also underlined the potential of the barium : calcium (Ba : Ca) ratio to track environmental histories of fish (Elsdon and Gillanders 2005; Hamer *et al.* 2006).

The purpose of the present study was to investigate the life-history traits of two ancestral gobioid species of the family Rhyacichthyidae (*P. attiti* and *R. guilberti*) using (1) the otolith composition to validate the amphidromous life cycle of both species, and (2) the otolith microstructure to estimate the duration of the marine larval phase.

## Materials and methods

### Collection of specimens and otolith preparation

In total, 30 *Rhyacichthys guilberti* individuals were caught. Twenty-three *R. guilberti* individuals were caught from Vanuatu between November 2006 and January 2010, and seven specimens were caught from Papua in November 2010 (Fig. 1, Table 1). Sample size was limited by the declining population status and the field constraints. *P. attiti* is a New Caledonian endemic species exclusively found in rivers on peridotite beds that occur in the southern part of the island (Lord and Keith 2006) where several catchment areas were sampled in November 2008 (Table 1). As *P. attiti* is endangered and rare, only four specimens (2 juveniles, 2 adults) were sampled in New Caledonia (Table 1). All specimens were collected by electro-fishing

**Table 1.** Sampling sites and standard length ( $\pm$  s.d.) of specimens

Species	Archipelago	Island	River	<i>n</i>	Standard length (mm)
<i>Rhyacichthys guilberti</i>	Vanuatu	Santo	Pae River	2	48.3 $\pm$ 21.2 (27.8–106.3)
	Vanuatu	Malekula	Lasumwé River	21	
	Papua	Papua	Kumafa	7	42.3 $\pm$ 21.9 (29.1–90.9)
<i>Protogobius attiti</i>	New Caledonia		Lost coast	4	38.5 $\pm$ 18.8 (21.0–58.5)

equipment (Portable Deka 3000 electric device, DekaMarsberg, Germany) and a large hand-net. Fish were killed using an overdose of clove essential oil (10%) and kept in 95% alcohol. The standard length (SL) of each juvenile and adult fish was measured to the nearest tenth of a millimetre (mm) with a digital dial calliper (Mitutoyo, Aurora, IL) (Table 1).

*Sagitta* otoliths were extracted, carefully rinsed, dried and embedded in epoxy resin (Araldite 2020, Escil, Chassieu, France). They were ground along a transverse section by using sandpapers (400–2000 grains per inch) to expose the edge, and then polished with abrasive powder (grain diameter 0.5–3.0  $\mu$ m). The otolith sections were observed under an Olympus BX51 (Olympus, Tokyo) light microscope and an Olympus DP20 digital camera ( $\times$ 40–200 magnification).

#### Microchemistry analysis

Six otoliths of *R. guilberti* (3 from Vanuatu and 3 from Papua) and four otoliths of *P. attiti* from New Caledonia were analysed with an IR 1030 nm femtosecond laser (Alfamet-Novalase, Bordeaux, France) coupled to an Elan DRC II (Perkin Elmer, Shelton, CT) inductively coupled plasma–mass spectrometer (ICP-MS). Ablation conditions were as described by Tabouret *et al.* (2011) for the analysis of Gobiidae species. The ablation started 70  $\mu$ m before the nucleus and ended at the otolith edge passing through the core and the metamorphosis check mark. Quantification was allowed using the following three certified reference glasses: NIST614, NIST612 and NIST610 (National Institute of Standards and Technology, USA). Sr and Ba were standardised to calcium (i.e. Sr:Ca) on the basis of the stoichiometry of Ca carbonate (380 000  $\mu$ g Ca g<sup>-1</sup> otolith) (Campana 1999). The average detection limits based on three standard deviations (s.d.) of the blank gas were 3.28  $\mu$ g g<sup>-1</sup> for <sup>86</sup>Sr and 0.09  $\mu$ g g<sup>-1</sup> for <sup>138</sup>Ba, i.e. Sr:Ca and Ba:Ca ratios of 0.86  $\times$  10<sup>-5</sup> and 0.02  $\times$  10<sup>-5</sup>, respectively.

#### Ageing

According to previous validations of the daily growth of the otolith for amphidromous gobies (Yamasaki *et al.* 2007; Iida *et al.* 2010; Taillebois *et al.* 2012), it was assumed that the increments for the two Rhyacichthyidae species were formed on a daily basis. The first increment after the nucleus is assumed to occur at hatching (Lecomte-Finiger 1999), whereas the formation of the first daily increment generally starts when the larvae mouth opens and it is able to ingest exogenous food (Panfili *et al.* 2002; Keith *et al.* 2008). The number of daily increments on each otolith of *R. guilberti* (*n* = 30) and *P. attiti* (*n* = 4) was independently counted by two readers, from the nucleus to the recruitment check mark.

#### Statistical analysis

The data were statistically processed using the free R software (R Development Core Team 2011). First, the normality of the data was systematically verified using Shapiro test. The consistency of the results between the two readers was tested using Wilcoxon's paired test. The difference in the PLD among sampling localities for *R. guilberti* was tested by a Student's *t*-test for *R. guilberti*. Statistical differences of the larval duration from Vanuatu and Papua for *R. guilberti* were tested using Man–Whitney *U*-tests.

## Results

### Microchemistry

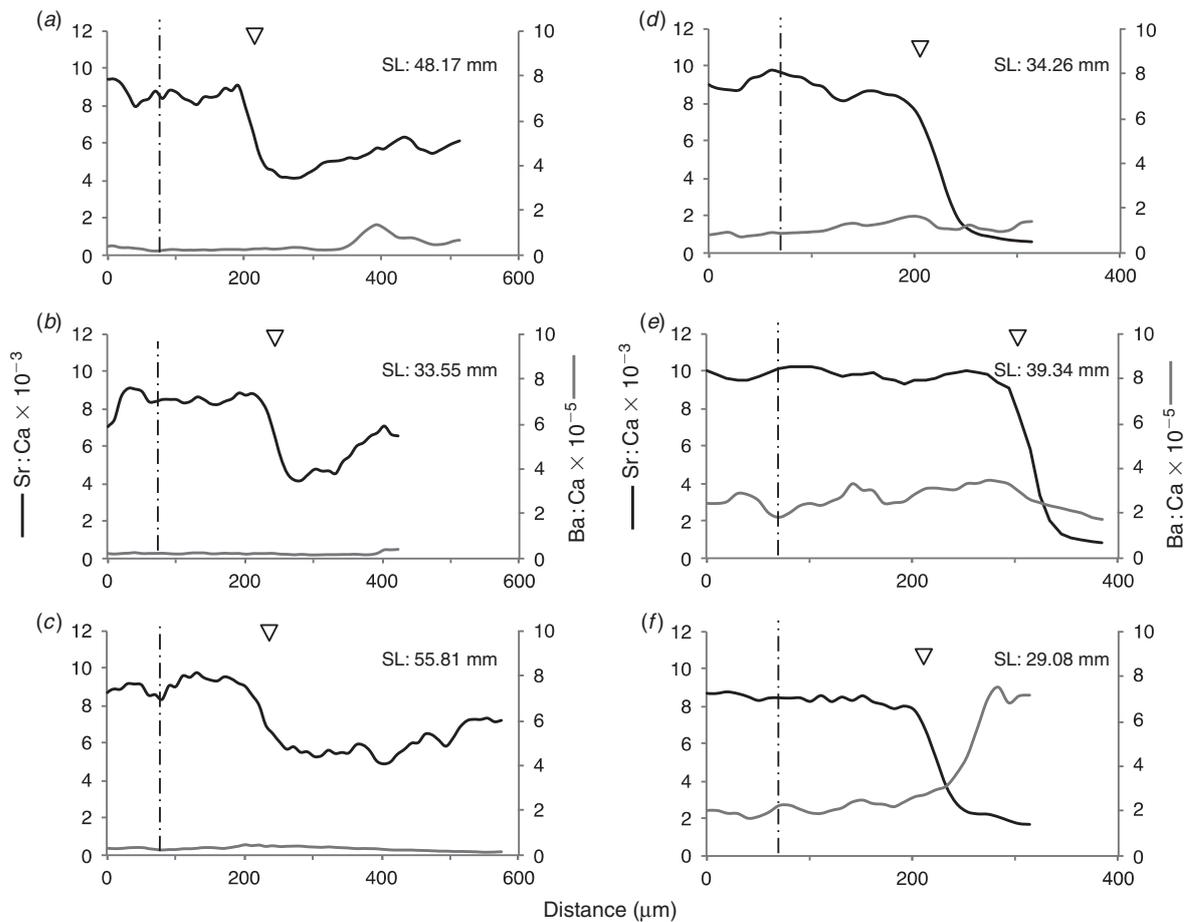
#### Rhyacichthys guilberti

From the LA-ICP-MS, all the *R. guilberti* otoliths studied (*n* = 6) showed similar patterns for the Sr:Ca ratio (Fig. 2). During the larval phase, i.e. from the nucleus to the check mark, the Sr:Ca ratio was high and constant, with mean values ( $\pm$  s.d.) of 8.74  $\pm$  0.51  $\times$  10<sup>-3</sup> and 9.22  $\pm$  0.77  $\times$  10<sup>-3</sup> for the individuals from Vanuatu and Papua, respectively. At the check mark, the Sr:Ca decreased drastically and reached a significant lower value (Student's *t*-test, *P* < 0.001) of 5.63  $\pm$  0.84  $\times$  10<sup>-3</sup> for Vanuatu specimens and of 1.60  $\pm$  0.88  $\times$  10<sup>-3</sup> for Papua specimens.

In the otolith of the specimens from Vanuatu, the Ba:Ca ratios were very low between the nucleus and the check mark (0.26  $\pm$  0.05  $\times$  10<sup>-5</sup>). For two specimens (Fig. 2*b, c*), the Ba:Ca ratio was not significantly different before and after the check mark (Student's *t*-test, *P* = 0.558) and stayed at low levels (0.26  $\pm$  0.09  $\times$  10<sup>-5</sup>) until the otolith edge. For one of the three Vanuatu specimens, an increase in Ba:Ca was observed, with values four-fold higher than the Ba:Ca background values (Fig. 2*a*). In the otoliths of Papua fish, the Ba:Ca ratio between the nucleus and the check mark was significantly higher (2.19  $\pm$  0.77  $\times$  10<sup>-5</sup>; *P* < 0.001) than that in the Vanuatu ones. After the check mark, two patterns were observed. For two individuals (Fig. 2*d, e*), the Ba:Ca ratio did not vary and stayed at a low level (1.89  $\pm$  0.85  $\times$  10<sup>-5</sup>). For the third individual (Fig. 2*f*), the Ba:Ca ratio showed a pattern opposite to that of the Sr:Ca ratio and markedly increased after the check mark, until values  $\sim$  7  $\times$  10<sup>-5</sup>.

#### Protogobius attiti

The Sr:Ca and Ba:Ca profiles observed in the *P. attiti* otoliths were more complex than those in *R. guilberti* (Fig. 3). In the area located 20  $\mu$ m around the nucleus, the mean value of the Sr:Ca ratio was 8.41  $\pm$  0.54  $\times$  10<sup>-3</sup> and that of the Ba:Ca



**Fig. 2.** Profiles of strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) ratios ( $\mu\text{g g}^{-1}$  provided by femtosecond laser ablation coupled to an inductively coupled plasma–mass spectrometer (fs-LA-ICP-MS) on otoliths of three adults of *Rhyacichthys guilberti* from (a–c) Vanuatu and (d–f) Papua. Nucleus (dotted line) and check mark (white triangle) are shown. SL, standard length (mm).

ratio was  $0.32 \pm 0.04 \times 10^{-5}$  for all the individuals. At 50–100  $\mu\text{m}$  after the nucleus, Sr:Ca ratios decreased progressively until the end of the profiles (Fig. 3a, c, d), except for one of the juveniles that showed Sr:Ca as high as at the nucleus (Fig. 3b). For both adults and one juvenile (Fig. 3a, c, d), the Sr:Ca reached values below  $6 \times 10^{-3}$  at the end of the profile. At the check mark, the decrease in the Sr:Ca ratio was followed by a progressive increase in the Ba:Ca ratio. The Ba:Ca ratio was highly variable and reached values between  $0.5 \times 10^{-5}$  and  $2.5 \times 10^{-5}$  at the end of the profile.

#### Estimation of the age at recruitment (pelagic larval duration)

##### *Rhyacichthys guilberti*

There was no significant difference in the age at recruitment between the two readers (Wilcoxon paired test;  $t = -0.992$ ,  $P = 0.325$ ). Mean PLD ( $\pm$  s.d.) was  $26.8 \pm 0.7$  days for the specimen caught in Vanuatu ( $n = 23$ ) and  $34.1 \pm 2.2$  days for the specimen caught in Papua ( $n = 7$ ). Normality and homoscedasticity were verified for PLD of *R. guilberti*. There was a significant difference in PLD between Vanuatu and Papua (Student's  $t$ -test,  $P = 0.005$ ).

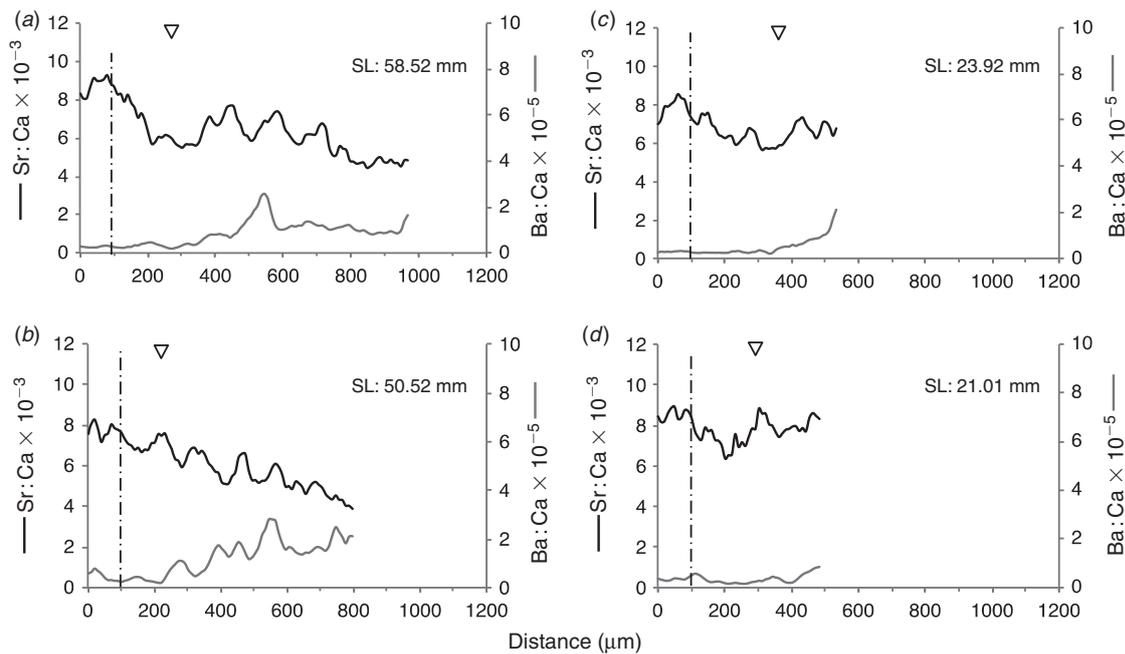
##### *Protogobius attiti*

The PLD for the four *P. attiti* fish caught in the New Caledonian river was variable. PLD was 60 and 69 days for juveniles, and 37 and 55 days for adults (mean  $\pm$  s.d.:  $55.2 \pm 13.5$  days).

## Discussion

### Amphidromy

The present study is the first investigation of the migratory behaviour using otolith microchemistry for the two ancestral gobioids, *R. guilberti* and *P. attiti*. The analysis of *R. guilberti* otolith revealed high Sr:Ca ratios and low Ba:Ca values from the nucleus to the check mark, i.e. the larval phase. Elevated Sr:Ca ratios in fish otoliths are widely accepted as proof of occupation of marine habitats and these values are consistent with those found during the marine larval phase of species of amphidromous goby (Radtke and Kinzie 1996; Shen *et al.* 1998; Tsunagawa *et al.* 2010), especially Sicydiinae (Table 2; Tabouret *et al.* 2011; Lord *et al.* 2011; Taillebois 2012). In otoliths of *R. guilberti* from Vanuatu, the Sr:Ca ratio decreased at the check mark, to reach values  $\sim 5 \times 10^{-3}$ . Same values were



**Fig. 3.** Profiles of strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) ratios ( $\mu\text{g g}^{-1}$ ) provided by femtosecond laser ablation coupled to an inductively coupled plasma–mass spectrometer (fs-LA-ICP-MS) on otoliths of two (a, b) post-larvae of *Protogobius*, and (c, d) two adults from New-Caledonia. Nucleus (dotted line) and check mark (white triangle) are shown. SL, standard length (mm).

**Table 2.** Strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) ratios in otolith of Rhyacichthyidae, Sicydiinae and other amphidromous goby species from insular systems

Species	Location	Larval phase		Post-recruitment		Reference
		Sr:Ca ( $\times 10^{-3}$ )	Ba:Ca ( $\times 10^{-5}$ )	Sr:Ca ( $\times 10^{-3}$ )	Ba:Ca ( $\times 10^{-5}$ )	
<i>Rhyacichthys guilberti</i>	Vanuatu	$8.74 \pm 0.51$	$0.26 \pm 0.05$	$5.64 \pm 0.84$	$0.37 \pm 0.26$	This study
	Papua	$9.22 \pm 0.77$	$2.19 \pm 0.75$	$1.60 \pm 0.88$	$3.04 \pm 2.15$	This study
<i>Protogobius attiti</i>	New Caledonia	$8.41 \pm 0.54$	$0.32 \pm 0.04$	6	2–5	This study
<i>Sicyopterus lagocephalus</i>	Vanuatu	10–12	<1	2–5	<1	Lord et al. (2011)
	New Caledonia	10–12	<1	2–4	0.4–0.8	Lord et al. (2011)
<i>Sicyopterus aiensis</i>	Vanuatu	10–12	<1	2–5	<1	Lord et al. (2011)
	New Caledonia	10–12	<1	2–4	0.4–0.8	Lord et al. (2011)
<i>Sicyopterus zosterophorum</i>	Vanuatu	$9.55 \pm 0.69$	$0.63 \pm 0.41$	$5.30 \pm 0.52$	$0.53 \pm 0.18$	Taillebois (2012)
	Papua	$9.25 \pm 0.64$	$0.88 \pm 0.50$	$5.26 \pm 0.75$	$0.95 \pm 0.52$	Taillebois (2012)
<i>Sicydium punctatum</i>	Guadeloupe	$9.39 \pm 0.50$	$0.55 \pm 0.16$	$3.23 \pm 0.48$	1–3	Tabouret et al. (2011)
<i>Rhinogobius</i> spp.	Honshu–Shikoku (Japan)	6–7		0.99–2.76		Tsunagawa and Arai (2008)

observed in freshwater post-recruitment phase in *Sicyopterus lagocephalus*, *S. aiensis* (Lord et al. 2011) and *Sicyopus zosterophorum* (Taillebois 2012) from Vanuatu. The lowest Sr:Ca ratios were found in Papua specimens after the check mark. These ratios are consistent with values given by Tsunagawa et al. (2010) for the post-recruitment phase of *Rhinogobius* spp. from rivers in Japan and also by Lord et al. (2011) for *S. lagocephalus* and *S. sarasini* from rivers in New Caledonia. The succession of a larval phase in a marine environment and a settlement in freshwater habitats validate the amphidromous biological life cycle of *R. guilberti*.

The elemental profiles from the *P. attiti* otolith seemed to indicate a progressive habitat change during their life cycle. The Sr:Ca ratios around the nucleus were similar to those obtained for *R. guilberti* and other amphidromous species during the marine larval phase (Table 2). The juvenile profiles may suggest that the fish stayed in a marine and/or estuarine environment until the check mark. However, contrary to the other amphidromous species studied, the observed decrease of Sr:Ca was progressive and not drastic. The simultaneous Ba:Ca increase and the Ba:Ca values observed at the end of the elemental profile that were similar to those found in freshwater system in

New Caledonia and Guadeloupe (Tabouret *et al.* 2011; Lord *et al.* 2011), may illustrate the fish movement to the freshwater habitat where they were caught. Moreover, high Sr : Ca ratios in freshwater are not uncommon and have already been observed in other systems (Gillanders, 2005) and suspected on this coast (Lord *et al.* 2011). Further investigations of the Sr and Ba behaviour along the river continuum are required as are investigations on metamorphosis and larval development according to the salinity gradient, to clarify the biological cycle of the species.

#### Pelagic larval duration

For both *R. guilberti* and *P. attiti*, the mean larval duration was quite similar to that of many reef fish (30–50 days; Victor 1986b; Wellington and Victor 1989; Wilson and McCormick 1999) and marine gobies (30–45 days; Brothers *et al.* 1983; Sponaugle and Cowen 1994; Shafer 2000). On the contrary, the PLD of these two species was shorter than for many other amphidromous gobies. For *Sicyopterus japonicus* (Tanaka, 1909), ranging from Taiwan to central Japan, the larval duration was estimated to be  $163.7 \pm 12.8$  days (Shen and Tzeng 2008) and 173–253 days (Iida *et al.* 2008); for *S. lagocephalus*, *S. aiensis* and *S. sarasini*, Lord *et al.* (2010) estimated the PLD at  $131 \pm 3.4$  days,  $79.2 \pm 4.6$  days, and  $76.5 \pm 3.9$  days, respectively. According to Maeda *et al.* (2007, 2011), long PLD may reflect sparseness of the habitat of those amphidromous gobioids (insular freshwater streams) compared with coastal fish, as colonisation to the sparsely distributed habitats might require greater dispersal ability over a longer PLD. Nevertheless, Taillebois *et al.* (2012) found a shorter PLD of nearly 54 days on one widespread Pacific amphidromous Sicydiinae (Gobiidae). The long duration of the oceanic larval life probably reflects the species ability to delay metamorphosis, so as to find suitable growth areas (Victor 1986a; Keith *et al.* 2008), locate isolated freshwater settlement sites and colonise new islands (Radtke *et al.* 2001; Murphy and Cowan, 2007; Taillebois *et al.* 2012). *R. guilberti* and *P. attiti* may not have the same plasticity. The lack of variation of PLD observed in *R. guilberti* suggests that this species cannot delay the metamorphosis as much as do other freshwater gobies such as *Sicyopterus* species. On the contrary, the variation of PLD is larger in *P. attiti*, and suggests a higher plasticity; however, this has to be confirmed with a larger sampling.

Differences in PLD and variability differences observed between both species in the present study constitute a new paradox in the amphidromous cycle and endemism processes because it was formerly considered that longer and variable PLD was related to a wider distribution range of species (Lord *et al.* 2010). However, Taillebois *et al.* (2012) showed that for *Sicyopus* and *Smilosicyopus* species (Sicydiinae), widespread and endemic species have the same PLD. However, the link between the PLD and species distribution is complex. The larval duration is influenced by the diet availability, and the salinity and temperature of the water (Bradbury and Snelgrove 2001; Radtke *et al.* 2001). Furthermore, the species distribution and dispersal abilities are also linked to endogenous factors, physical processes and biogeographic and historical factors (Keith *et al.* 2005; Lord *et al.* 2010).

*Protogobius attiti* is a species found only in ultramafic rivers in the south of New Caledonia. Despite a longer PLD than for

*R. guilberti*, the environmental requirements for this species restrict its natural distribution to this type of rivers, which are particularly limited in the area (Lord and Keith 2006). The high variability of its PLD could reflect the ability, for this species, to wait for the best conditions to recruit in the rivers. Studies on eggs, yolk-reserve and distribution pattern in the ocean or coastal waters would be the next key step for a better understanding of the pelagic larval phase, the dispersal abilities of endemic and widespread species and the evolution of the amphidromous life cycle. This is of a particular interest to understand the species resilience and propose reliable tools for the management and conservation of patrimonial species. This may support the restoration and conservation of ecological corridors.

#### Acknowledgements

First, we thank all partners that financially supported this work, namely the UMR BOREA 7208 (MNHN-CNRS-IRD-UPMC), the New Caledonian Government, the National Museum of Natural History of Paris (ATM bio-minéralisation) and the Fondation de France. We also thank the Vanuatu Environment Unit (D. Kalfatak; Permit Numbers ENV326/001/1/07/DK and ENV326/001/1/08/D) and the New Caledonian North and South Provinces (J-J. Cassan, J. Manauté and C. Méresse) for allowing sampling (Permit Number 1224-08/PS). We are especially grateful for the Lengguru Expedition (Papua; Permit Number 43/SI/M26/X/2010) to: Muhfizar, Director of Akademi Perikanan Sorong (APSOR) and the team of Vessel Airaha; Kadarusman (APSOR); Ruby V. Kusumah and Gigih S. Wibawa (Balai Riset Budidaya Ikan Hias-BRBIH Depok-KKP); Laurent Pouyau, and all of the scientific team; Chiefs of tribes and villagers in the region; and P. Gaucher, C. Flouhr and G. Ségura for their assistance on the field.

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