

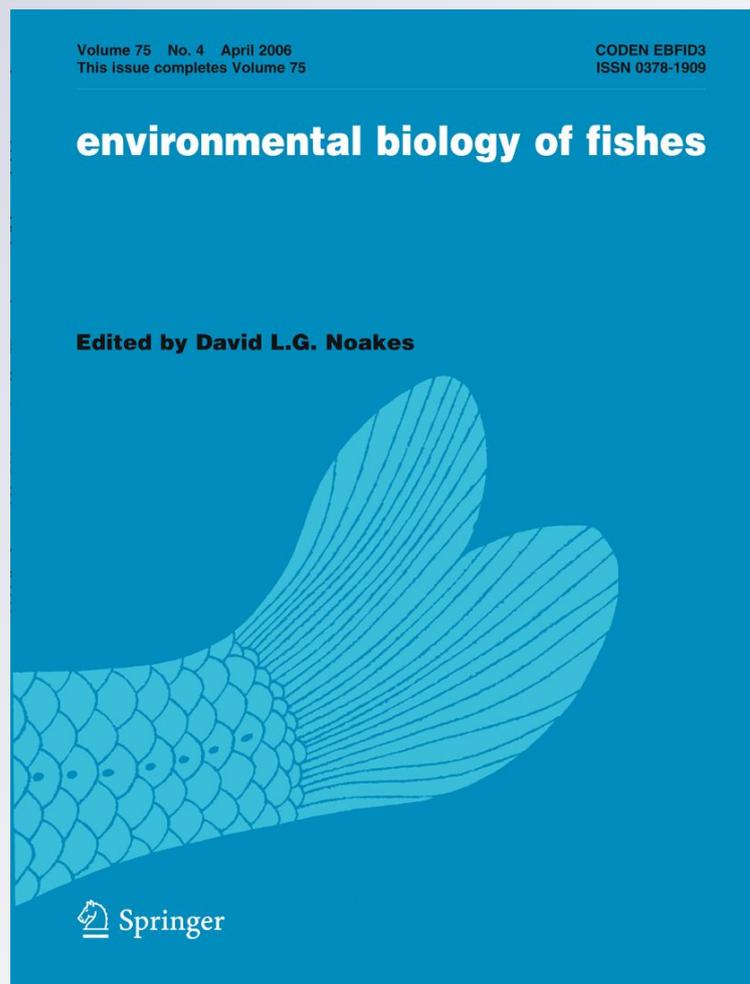
*Otolith shape analysis for three
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Otolith shape analysis for three *Sicyopterus* (Teleostei: Gobioidae: Sicydiinae) species from New Caledonia and Vanuatu

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Abstract Otolith shape analysis has been used in a number of studies as an inexpensive and powerful method for categorising fish in individual stocks. Elliptical Fourier analysis was used on three different amphidromous *Sicyopterus* species. *Sicyopterus lagocephalus* is a widespread species while the other two have a limited distribution area, *Sicyopterus aiensis* being endemic to Vanuatu, and *Sicyopterus sarasini* to New Caledonia. Both endemics live in sympatry with

the widespread species. The otolith shape of all fish sampled was a clear species differentiator, thereby demonstrating that otolith shape is species-specific. At an intraspecific level there are different river populations within samples from Vanuatu, indicating a western group and an eastern “central” group. These results are congruent both for the endemic species, *S. aiensis* and for the cosmopolitan species. Finally, we found that, for *S. lagocephalus*, the cosmopolitan species, New Caledonian samples are close to western Vanuatu samples, the latter two being well differentiated from the eastern “central” Vanuatu samples. The explanation for these results may lay either in the influence of environmental factors on the otolith shape, or in the influence of common early life history thus reflecting genetic factors, or a combination of both.

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Introduction

Pacific island rivers are home to amphidromous gobies which represent most of the biodiversity of these ecosystems (Keith 2003). Their amphidromous life cycle is an adaptation to life in insular river systems (McDowall 2007), which are generally oligotrophic environments subject to extreme hydrological and climatic seasonal variations (Keith 2003). The adults feed, grow and reproduce in rivers. After

hatching, larvae undergo a downstream migration towards the sea where they spend three to six months (Hoareau et al. 2007; Lord et al. 2010). After this marine phase, which is supposedly a key element in the dispersion of these species (McDowall 1997, 2007), post-larvae return to rivers. During their recruitment, they undergo an important metamorphosis enabling them to shift from a pelagic feeding mode to a benthic feeding mode and to migrate upstream towards the adult habitats (Keith et al. 2008). The *Sicyopterus* genus is the most diversified of these amphidromous gobies. Adults live in clear and well-oxygenated streams, feeding on diatoms and algae, scraping them off the rocky substrate (Marquet et al. 2003; Keith et al. 2008; Lord and Keith 2008).

In the New Caledonia/Vanuatu region, there are three *Sicyopterus* species. The first, *S. lagocephalus*, is a cosmopolitan species, as its distribution area ranges from the Western Indian Ocean to the Eastern Pacific (Watson et al. 2000). The other two species are endemics, *S. aiensis* from Vanuatu (Keith et al. 2010) and *S. sarasini* from New Caledonia (Marquet et al. 2003). Both endemics live in sympatry with the cosmopolitan species. Finally, *S. sarasini* only occurs in the south of New Caledonia, on nickel-rich ultramafic substrate (Lord and Keith 2008).

Otoliths, calcium carbonate structures located in the fish's inner ear, are an indirect means for studying fish populations and assessing the relationship between the environment and the organisms. Given the abundance of studies, the shape of the otolith would appear to be an ideal marker for fish populations (Smith 1992; Campana and Casselman 1993; Tracey et al. 2006; Morat et al. 2008). Otolith shape has long been known to be species specific (L'Abbé-Lund 1988; Aguirre and Lombarte 1999; Parmentier et al. 2001) and less variable in growth than fish growth, presumably because of the fact that otoliths are organs contributing to audition and balance (Campana and Casselman 1993; Popper et al. 2005). Otoliths are considered to "record" the fish's life (Lecomte-Finiger 1999): they grow throughout the life of the fish and are a metabolically inert structure; once deposited, otolith material is unlikely to be resorbed or altered (Campana and Neilson 1985; Casselman 1987). Otoliths are therefore an effective tool for studying fish populations.

Elliptical Fourier functions are often used as they represent a precise method for describing and charac-

terising outlines, efficiently capturing outline information in a quantifiable manner (Kuhl and Giardina 1982; Lestrel 1997; Tracey et al. 2006). Furthermore, this technique may be an inexpensive way for studying populations, and may provide new insight for conservation and management measures.

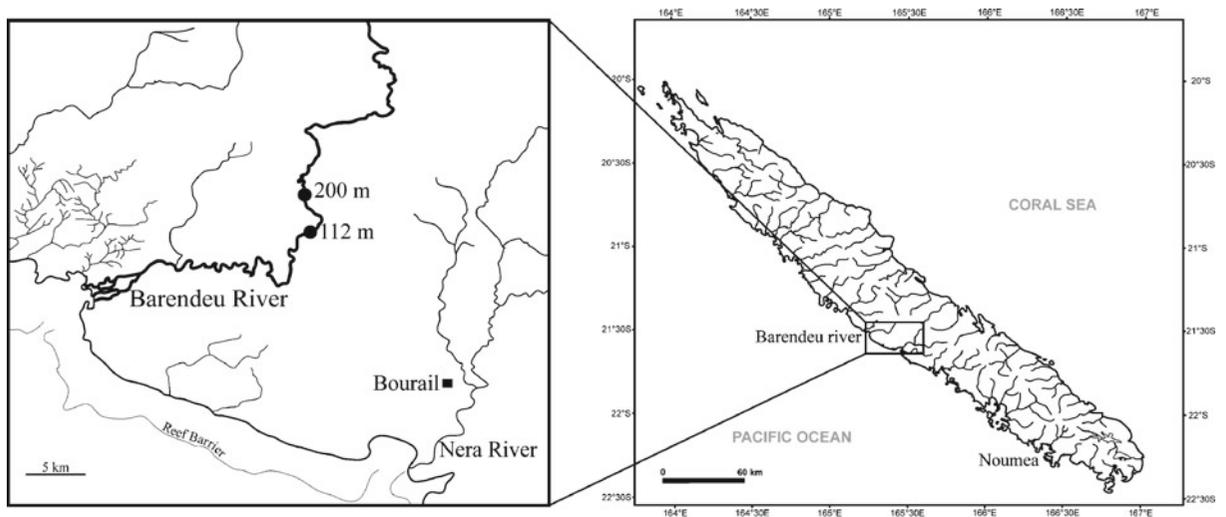
In this study, we tested the hypothesis that (i) the otolith shape can be used to distinguish the three *Sicyopterus* species of the New Caledonia/Vanuatu region, that (ii) otolith shape can be used to differentiate between *Sicyopterus* populations, and finally that (iii) the results are congruent between different species.

Materials and methods

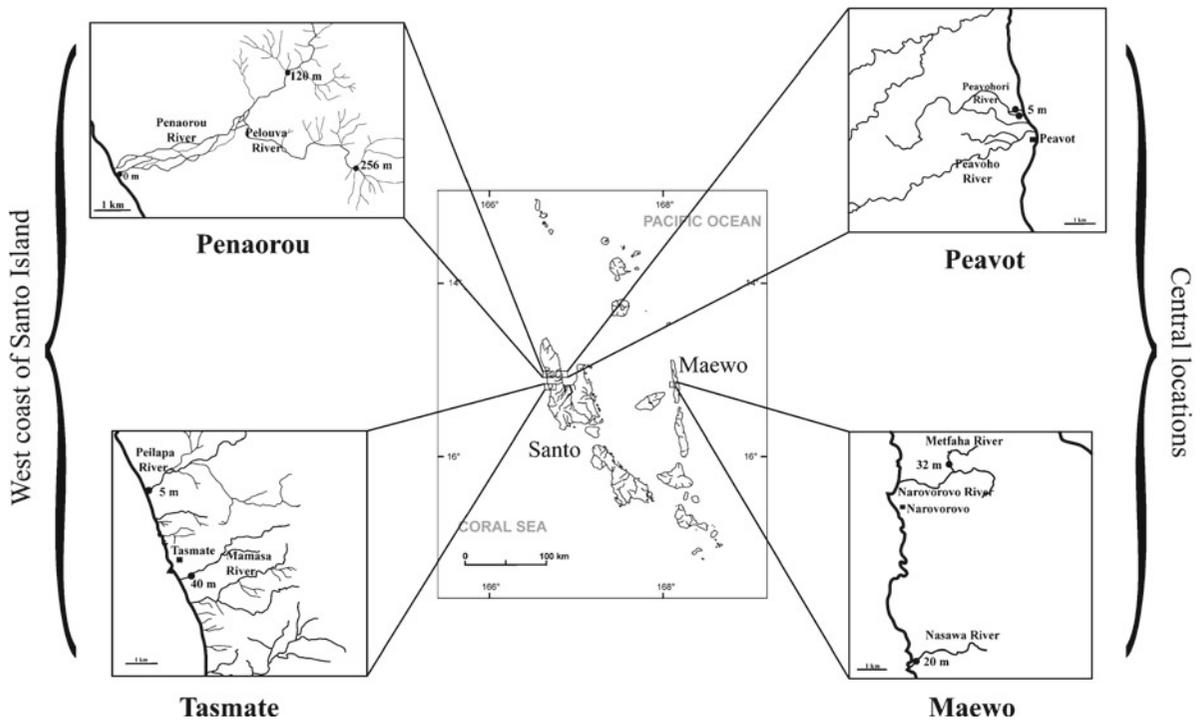
Study areas and sampling

New Caledonia is located 1500 km east of the East Australian coast at the border of the Melanesian arc in the Southwest Pacific Ocean. Its geographic position is between latitude 20°–30°S and longitude 164°–168°E (Keith et al. 2009). It comprises one major island called "Grande Terre" and small islands, the Loyalty Islands. Unlike most Pacific islands, New Caledonia is of continental origin, as it was once part of the Gondwanaland Continent. Surveys in New Caledonia were performed between June 2006 and November 2007. All samples of both *S. sarasini* and *S. lagocephalus* from New Caledonia were caught in the Barendu River (Fig. 1a). This river is located on the mid-West coast of Grande Terre and on ultramafic substrate, i.e., substrate from which nickel is extracted. It is fast flowing and well oxygenated. The bottom of the river is covered in rocks and large boulders, offering many hiding places for *Sicyopterus* species.

Vanuatu is located to the northeast of New Caledonia, between latitude 13°–20°S and longitude 166°–170°E. It comprises about 80 islands of volcanic origin, which appeared between 22 million years ago (Ma) for the oldest islands (Santo and Malakula) and less than 5 Ma ago (Green et al. 1988). The various surveys were carried out in November 2006 on Santo Island, and in November 2007 on Maewo Island. Santo is the largest and also the oldest of the archipelago's islands (22 Ma). It is a volcanic island, although it also shows calcareous areas of coralline



(a) New Caledonia



(b) Vanuatu

Fig. 1 Sampling location in New Caledonia (a), i.e., the Barendu River on the central West coast. Sampling locations in Vanuatu (b) showing the rivers on the West coast of Santo Island (Penaorou and Tasmate) and the “central loations” (Peavot and Maewo)

origin. Rivers on Santo are short and fast flowing, presenting a perfect biotope for Sicydiinae gobies (Keith 2003). Maewo Island is younger (5 Ma), and has a large chain of mountains running through its

centre. Maewo reports Vanuatu’s highest level of precipitation, explaining the presence of large waterfalls, which are favourable habitats for Sicydiinae gobies, especially *Sicyopterus* species (Keith et al.

2004; Keith et al. 2009). The endemic species of Vanuatu, *Sicyopterus aiensis*, was caught in the three catchment areas that were surveyed in Santo, Tasmate (2 rivers, Mamasa and Peilapa Rivers), Penaorou (1 river, Penaorou River) and Peavot (1 river, Peavohori River), the first two being on the West side of the Cumberland Cape and the third on the east side of the Cumberland Cape. *S. aiensis* was also found in Maewo (2 rivers, Nasawa and Matfaha Rivers) (Fig. 1b). These rivers sampled in Vanuatu all are the same type, i.e., short, fast-flowing, oxygenated rivers on volcanic substrate.

All specimens were sampled by electrofishing (Portable Deka 3000 electric device, Ltd Germany), combined with a large hand net. A total of 41 *Sicyopterus lagocephalus* and 22 *Sicyopterus sarasini* were caught in New Caledonia in the Barendeu River. A total of 95 *Sicyopterus lagocephalus* and 84 *Sicyopterus aiensis* were caught in Vanuatu (Table 1). All individuals were measured (standard length=SL); the size range is approximately the same for all the sites sampled. Different environmental parameters were measured in each river (Table 1).

Otolith extraction and shape analysis

As *sagittae* are the largest otoliths of the three pairs, they are the ones usually used for otolith analysis (Campana and Casselman 1993). *Sagittae* were extracted in the laboratory. Fish were dissected under a binocular magnifier (Olympus VMZ, Ltd Paris, France). The otoliths were placed in distilled water just after the extraction to clean them, eliminating remaining tissue from the *macula* and the vestibule with fine tweezers (Secor et al. 1992). Once cleaned, the otolith pairs were dried and kept dry in Eppendorf tubes, right and left otoliths were stored separately.

Each otolith was systematically placed with the *sulcus acusticus* facing the observer and was examined under a stereomicroscope fitted with a numerical camera (Sony XC-77CE CCD industrial Camera, Sony, France) linked to a computer (Fig. 2).

Digital images of each otolith were then acquired with the software VISILOG 6.3 (Noesis, Orsay, France) which also calculated the surface area of the otolith (A_0), its perimeter (P_0), its length (maximum measurement, L_0) and its width (maximum measurement, l_0) to the nearest 10^{-2} mm. These measurements were used to calculate five shape indices (Table 2), which are independent from differences in otolith size (Tuset et

al. 2003b). These measurements allowed us to assess whether there was a significant difference between the left and the right otolith of each individual. After verifying the normality of the data (Kolmogorov-Smirnov test, data not Normal, $p < 5\%$) and the homoscedasticity of the variances, we performed the non-parametric Wilcoxon signed-rank tests in order to see whether there were differences between the shape indices of left and right otoliths (SPSS v. 10, SPSS Inc. Headquarters, Chicago Illinois, USA).

The shape of each otolith was assessed with a Fourier series. Among several possibilities for analysing shape with Fourier series, the elliptic Fourier analysis (EFA) descriptors are often considered as more powerful than those derived from fast Fourier transformation (FFT) for shape analysis, and were therefore used in this work, although EFA is in fact less used than FFT (Stransky 2005; Stransky and MacLellan 2005; Tracey et al. 2006; Gonzalez-Salas and Lenfant 2007; Mériqot et al. 2007). This technique describes the outline with several components, named harmonics. Each harmonic is characterised by four coefficients, resulting from the projection of each point of the outline on axes (x) and (y). The higher the number of harmonics, the greater the accuracy of the outline description (Kuhl and Giardina 1982). For each numerical image, the software Shape 1.2 (Iwata and Ukai 2002) calculated the Fourier coefficients in order to make them invariant to the otolith size and its orientation (and position) with respect to the beginning of the outline, which is arbitrarily defined. In addition, the Fourier power (FP) spectrum was calculated in order to determine the minimum number of harmonics required for the best reconstruction of the otolith outline (Crampton 1995). The Fourier power of a harmonic is proportional to its amplitude and provides a measurement of the amount of "shape information" described by this harmonic. For the n^{th} harmonic, the Fourier power (FP_n) is given by the expression:

$$PF_n = \frac{A_n^2 + B_n^2 + C_n^2 + D_n^2}{2}$$

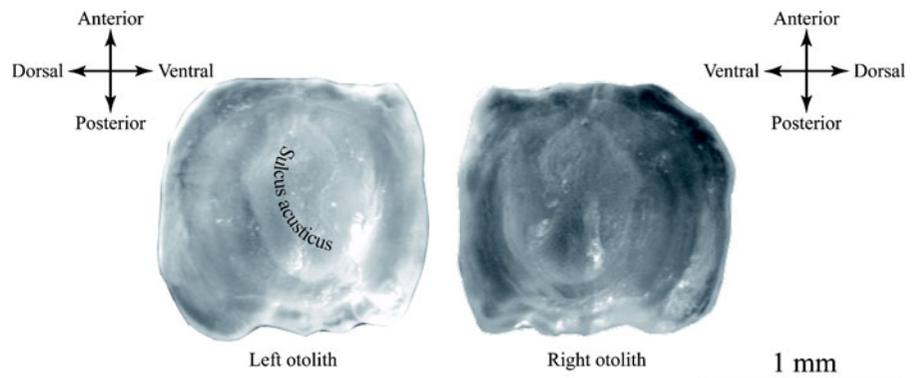
where A_n , B_n , C_n and D_n are the Fourier coefficients of the n^{th} harmonic.

The cumulated power percentage (PF_c) can then be defined by: $PF_c = \sum_1^n PF_n$ For this purpose, we randomly chose two sub-samples of 20 otoliths each (the first for right otoliths, the second for left) for the three

Table 1 Sampling locations in New Caledonia and Vanuatu for the three *Sicyopterus* species (N: number of samples) with the main characteristics of the sampling sites and the size range of the samples for each site (SL: standard length)

Species	Location	River	Latitude	Longitude	pH	Temperature (°C)	Conductivity (µS/cm)	Altitude (m)	Distance to river mouth (m)	N	Size range (SL)	Total
<i>S. sarasini</i>	New Caledonia	Baredeu	21°28'43"	165°23'30"	8.0	25.7	150	112	13000	22	46–100 mm	22
	New Caledonia	Baredeu	21°28'28"	165°23'26"	7.8	23.7	144	200	13000	16	44–67 mm	16
	New Caledonia	Baredeu	21°28'44"	165°23'30"	8.0	25.7	150	112	13000	25	39–77 mm	41
<i>S. laquecephalus</i>	Vanuatu	Mamasa	15°13'30"	166°40'4"	8.4	28.0	308	40	2000	24	34–55 mm	24
	Vanuatu	Penaorou	14°57'36"	166°37'59"	8.3	24.0	372	119	5250	7	35–53 mm	7
	Vanuatu	Penaorou	14°58'39"	166°35'56"	8.6	31.0	292	0	200	1	45 mm	1
	Vanuatu	Pelouva	14°58'37"	166°38'41"	8.1	24.3	226	256	4320	4	40–46 mm	4
	Vanuatu	Piavohori	14°58'21"	166°47'17"	7.9	24.5	299	5	200	28	28–53 mm	28
	Vanuatu	Metfaha	15°10'50"	168°7'13"	8.0	25.5	250	32	1500	25	65–30	25
<i>S. aietensis</i>	Vanuatu	Nasawa	15°12'13"	168°6'45"	8.0	25.6	279	20	100	6	37–62 mm	95
	Vanuatu	Mamasa	15°13'30"	166°40'4"	8.4	28.0	308	40	2000	15	36–70 mm	15
	Vanuatu	Peilapa	15°10'49"	166°39'6"	8.4	24.0	266	5	200	13	30–100 mm	13
	Vanuatu	Penaorou	14°57'36"	166°37'59"	8.3	24.0	372	119	5250	7	59–84 mm	7
	Vanuatu	Penaorou	14°58'38"	166°35'56"	8.6	31.0	292	0	200	3	48–61 mm	3
	Vanuatu	Piavohori	14°58'21"	166°47'17"	7.9	24.5	299	5	200	32	34–90 mm	32
	Vanuatu	Metfaha	15°10'50"	168°7'13"	8.0	25.5	250	32	1500	13	50–79 mm	13
Vanuatu	Nasawa	15°12'13"	168°6'45"	8.0	25.6	279	20	100	1	76 mm	1	
												84

Fig. 2 Digital images of a *Sicyopterus aiensis* otolith pair (Santo Island, Tasmate, Peilapa River, SL: 100 mm). The *sulcus acusticus* is facing the observer



species; and the threshold of 99.99% of the mean cumulated Fourier power was chosen to define the adequate number of harmonics to be considered in the analyses. As the first 20 harmonics totalled 99.99% of the cumulated power for both right and left otoliths, the Fourier analysis indicated that the otolith shape of the studied species could be summarised by these 20 harmonics, i.e., 80 Fourier coefficients. However, the coefficients derived from the 1st harmonic were not taken into account, because the outline reconstructed with these coefficients is a simple ellipse resulting in a maximum Fourier power. These coefficients would then mask the information derived from the other harmonics (Crampton 1995). So, 19 harmonics and thus 76 Fourier coefficients were used for the data analyses.

Both right and left otoliths were used to define one individual; the data for both otoliths was then assembled and, as a result, a total of 152 variables were used to describe one individual.

Data analysis

Canonical discriminant analyses (CDA) were used to determine differences firstly between species, and secondly between sites at an intraspecific level. The

CDA were performed with the Fourier coefficients (19 harmonics per otolith, thus 152 coefficients). The objective of CDA is to investigate the integrity of pre-defined groups, i.e., individuals belonging to a given sample, such as species, geographical area or regional site, by finding linear combinations of descriptors that maximize the Wilks lambda (λ) (Ramsay and Silverman 2005; Pothin et al. 2006). The Wilks λ assesses the performance of the discriminant analyses. This statistic is the ratio between the intra-group variance and the total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted groups' membership. λ values range from 0 to 1: the closer λ is to 0, the better the discriminating power of the CDA.

Several CDA were performed. The first objective was to determine whether the three species studied, *S. lagocephalus*, *S. sarasini* and *S. aiensis*, could be discriminated on the basis of their otolith shape. Our second aim was to discriminate local populations of each species. The second analysis could only be performed on *S. aiensis* and *S. lagocephalus* as all *S. sarasini* were sampled in the same river. Rivers in Vanuatu were grouped in their catchment area to increase the sample size (Tasmate: Mamasa and Peilapa Rivers; Penaorou: Penaorou and Pelouva Rivers; Peavot: Piavohori River; and Maewo: Metfaha and Nasawa Rivers). Thus, the study was undertaken between the 4 catchment areas sampled in Vanuatu for the endemic species *S. aiensis* and for the cosmopolitan *S. lagocephalus*, and we looked at whether results were congruent between these two species. In the rest of the paper, Tasmate and Penaorou will be referred to the "western" location and Peavot and Maewo will be referred to the

Table 2 Shape indices established from morphometric measurements with A_0 , the surface area of the otolith; P_0 , the perimeter; L_0 , the length and l_0 , the width

Shape indices	Formula
Form factor	$(4\pi A_0)/P_0^2$
Roundness	$(4A_0)/(\pi L_0^2)$
Circularity	P_0^2/A_0
Rectangularity	$A_0/(L_0 \times l_0)$
Ellipticity	$(L_0 - l_0)/(L_0 + l_0)$

“central” location, as they are located toward the inside of the “V” formed by the various islands of the Vanuatu archipelago. Finally, the analysis was undertaken at a larger scale for *S.lagocephalus*, that is, between Vanuatu and New Caledonia.

The reclassifying rate of individuals within the pre-defined groups was determined with a Cohen-Kappa test (Titus et al. 1984). The Cohen-Kappa values were scaled up to a percentage.

Results

Shape indices

For the three species, the Wilcoxon signed-rank test showed that at least three of the shape indices were significantly different between the right and the left otolith (Table 3). The presence of significant differences between the right and the left otoliths justifies the use of both otoliths to define one individual.

Species discrimination

The discrimination between the three species was good and the Cohen-Kappa test revealed that 97% of the fish were correctly classified. The two discriminant functions were significant with Wilks λ being respectively 0.05 ($P < 0.05$) for function 1 and 0.312 ($P < 0.05$) for function 2. The first discriminant function of the CAD explains 70.3% of the variability and allows us to separate *S.lagocephalus* from the other two species, which are not clearly separated by this first function. The second function of the CAD explains 29.7% of the variability and allows us to discriminate the two endemic species. We can then conclude that the three species are correctly differentiated in this analysis (Fig. 3).

Intraspecific analysis: *Sicyopterus aiensis*

Within Vanuatu, we defined four groups each corresponding to one catchment area. Thus, we have Tasmate (two rivers) and Penaorou (one river) on the Western coast of Santo Island, Peavot (one river) on the Eastern coast of Santo Island and Maewo Island (two rivers) on the Western coast of the island. 100% of the individuals were classified in their pre-defined group (Fig. 4). All three discriminant functions were significant with Wilks λ being respectively $7.3E^{-5}$

($P < 0.05$) for function 1, 0.003 ($P < 0.05$) for function 2 and 0.061 ($P = 0.001$) for function 3. The first two discriminant functions explain most of the variability. Indeed, function 1 explains 54.4% of the variability and allows us to separate locations on the Western coast of Vanuatu (Penaorou and Tasmate) from locations oriented towards the centre of the archipelago (Peavot and Maewo). Function 2 explains 25.2% of the variability and separates Santo Island from Maewo Island. When plotting function 3 (explaining 20.4% of the variability) versus function 1 we observe approximately the same results (not represented).

Intraspecific analysis: *Sicyopterus lagocephalus* in Vanuatu

We used the same four groups for this species as for *S. aiensis*, i.e., Tasmate (one river), Penaorou (two rivers), Peavot (one river) and Maewo (two rivers). 100% of the individuals were classified in their predefined group (Fig. 5). The first two discriminant functions explain 91.9% of the variability. Both were significant with Wilks λ being respectively $1.06E^{-4}$ ($P < 0.05$) for function 1 and 0.007 ($P < 0.05$) for function 2. Function 1 explains 71.7% of the variability and allows us to separate locations on the Western coast of Vanuatu (Penaorou and Tasmate) from locations oriented towards the centre of the archipelago (Peavot and Maewo). Function 2 explains 20.2% of the variability and separates Santo Island and Maewo Island.

Thus, for both *S. aiensis* and *S.lagocephalus* the same pattern was observed. For the two species, Penaorou and Tasmate (West Santo) catchment areas were placed together according to the first discriminant function, explaining most of the variability, and Peavot (East Santo) and Maewo catchment areas were placed together. The second function discriminates the two islands, Santo and Maewo.

Intraspecific analysis: *Sicyopterus lagocephalus* at the New Caledonia/Vanuatu scale

For this analysis, Tasmate and Peanorou (West coast, Santo) were pooled as were Peavot and Maewo (central locations), as these localities were grouped on the first function in the previous analysis. We therefore have three predefined groups: New Caledonia (Barendeau River), Western coast of Vanuatu (Tasmate/Penaorou) and “central” localities in Vanuatu

Table 3 Results for the Wilcoxon signed-rank statistical test (Z). For each species, significantly different shape indices are in bold. R: right otoliths; L: left otoliths

		Form R - Form L	Roundness R - Roundness L	Circularity R - Circularity L	Rectangularity R - Rectangularity L	Ellipticity R - Ellipticity L
<i>S. aiensis</i>	Z	-1.111	-4.744	-7.45	-2.566	-4.051
	p-value	0.266	0	0.456	0.01	0
<i>S. sarasini</i>	Z	-2.484	-2.289	-2.451	-0.958	-2.906
	p-value	0.013	0.022	0.014	0.338	0.004
<i>S. lagocephalus</i>	Z	-1.531	-7.007	-1.494	-5.635	-5.8
	p-value	0.126	0	0.135	0	0

(Peavot/Maewo). 100% of the individuals were classified in their predefined groups (Fig. 6). Firstly, this result confirms that Tasmate and Penaorou samples are close in terms of the shape of their otoliths, and that Peavot and Maewo samples are also close in terms of the shape of their otoliths.

The two discriminant functions were significant with Wilks λ being respectively 0.004 ($P < 0.05$) for function 1 and 0.088 ($P < 0.05$) for function 2. The first discriminant function of the CAD explains 66.3% of the variability and allows us to separate “central” locations in Vanuatu from the other two locations, New Caledonia and Vanuatu West coast sampling

areas, which are not separated on this first function. The second function of the CAD explains 33.7% of the variability and discriminates all locations, New Caledonia, West coast and central Vanuatu.

Discussion

The three *Sicyopterus* species were well differentiated on the basis of otolith shape. This is not surprising as otolith shape is markedly species-specific (L'Abbé-Lund 1988) and this has been shown in other studies (L'Abbé-Lund and Jensen 1993; Tuset et al. 2003a;

Fig. 3 Canonical discriminant analysis achieved with elliptic Fourier coefficient for *Sicyopterus*. The discrimination is a function of the species (*S. lagocephalus*, *S. aiensis*, *S. sarasini*). For each species, the outline of one pair of otoliths is illustrated, but it is not necessarily representative of all otolith shapes of the group to which it belongs

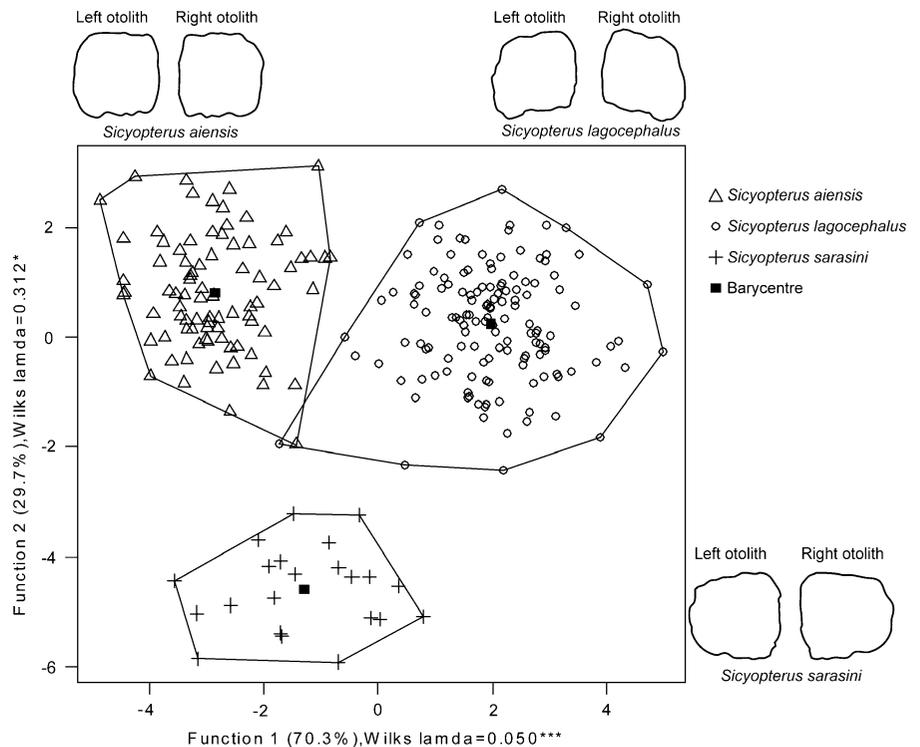
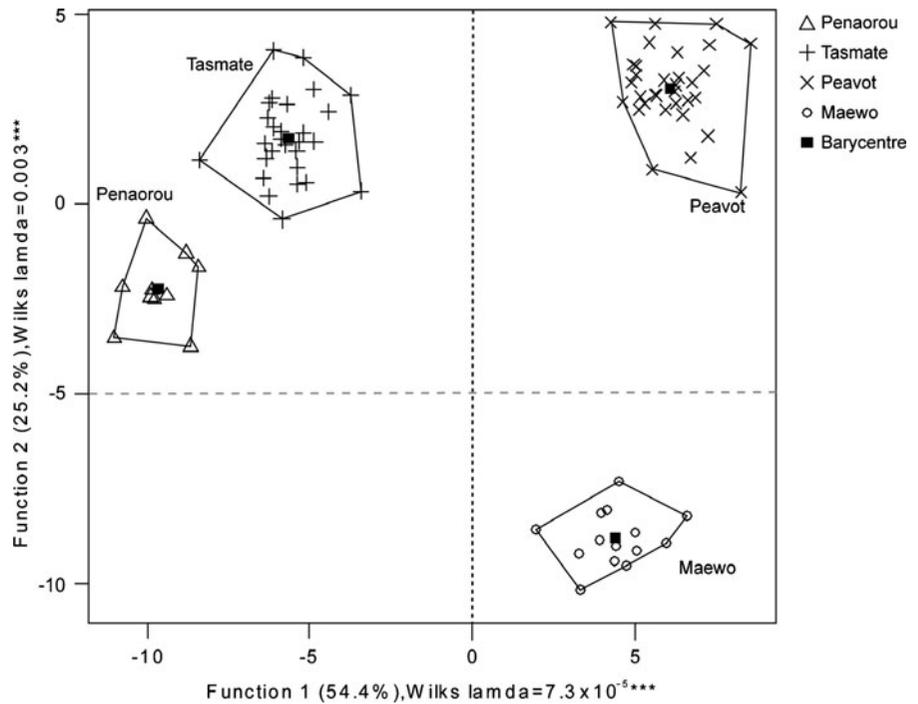


Fig. 4 Canonical discriminant analysis achieved with elliptic Fourier coefficients for *Sicyopterus aiensis*. The discrimination is a function of the sampling sites in Vanuatu. The vertical black dotted line shows the separation for the first function whereas the horizontal grey dotted line shows the separation for the second function



Tuset et al. 2006; Morat et al. 2008). The clear discrimination of the three species suggests that genetic effects control the overall otolith shape at a species level. Even though the three species are well differentiated in terms of their otolith shape, it seems that the

two endemic species, *S. sarasini* and *S. aiensis*, are closer to each other than to the widespread *S. lagocephalus*. Keith et al. (2005) have shown that *S. lagocephalus* may be the most recent species in the *Sicyopterus* genus. *S. sarasini* and *S. aiensis* may

Fig. 5 Canonical discriminant analysis achieved with elliptic Fourier coefficients for *Sicyopterus lagocephalus*. The discrimination is a function of the sampling sites in Vanuatu. The vertical black dotted line shows the separation for the first function whereas the horizontal grey dotted line shows the separation for the second function

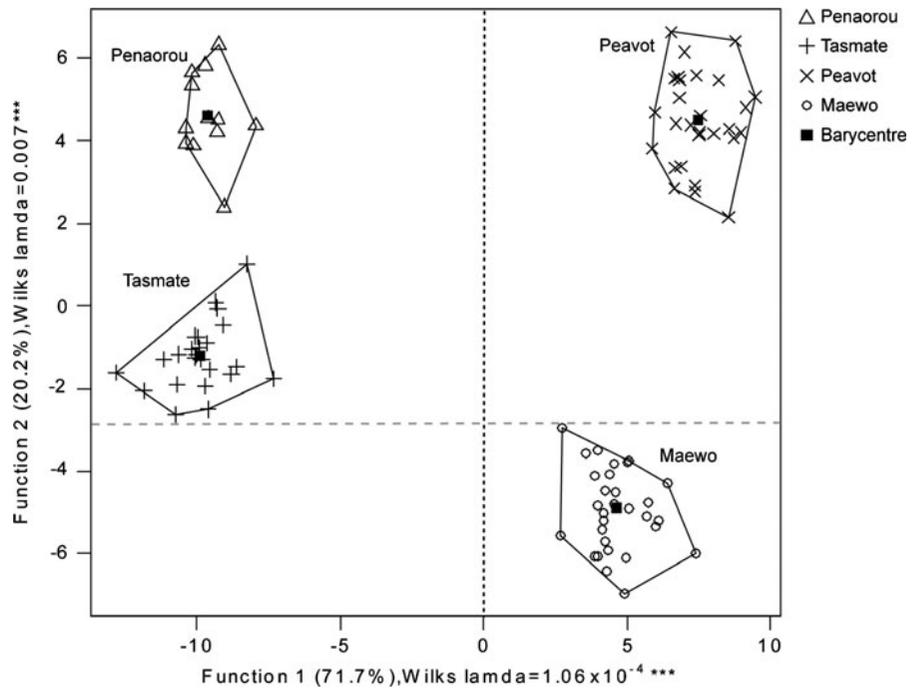
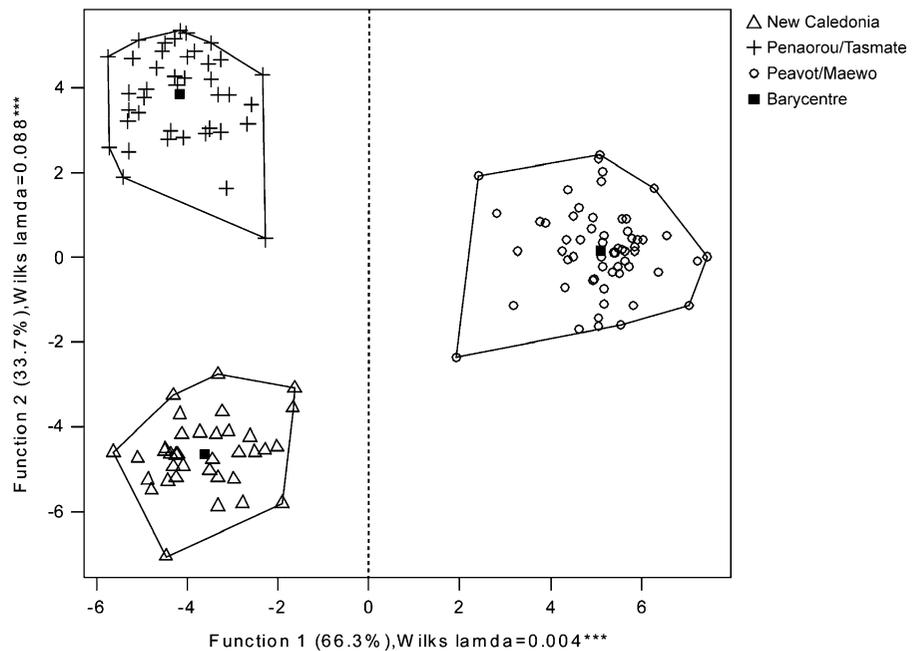


Fig. 6 Canonical discriminant analysis achieved with elliptic Fourier coefficients for *Sicyopterus lagocephalus*. The discrimination is a function of the sampling sites in New Caledonia and Vanuatu. The vertical black dotted line shows the separation for the first function



therefore be genetically closer to each other than to *S. lagocephalus*.

For both species fished in Vanuatu, *S. lagocephalus* and *S. aiensis*, otoliths of specimens caught on the West coast of Vanuatu seem to have similar shapes and are different from otoliths of specimens caught in the central locations. The fact that the results are congruent for the two species suggests that there are external driving forces acting on the otolith's shape, whatever the species. As, finally, for the study on *S. lagocephalus* between New Caledonia and Vanuatu where the specimens caught on the Western coast of Vanuatu and in New Caledonia were closer in terms of their otolith shape than to the samples caught in the central locations of Vanuatu.

Within a species, the otolith shape may vary according to geographical site and/or environmental factors (Hoff and Fuiman 1993; Lombarte and Leonart 1993; Begg et al. 2001; De Vries et al. 2002; Cardinale et al. 2004; Tuset et al. 2006) such as differences in substrate, food availability and type, niches, river flow, current speed (Vignon and Morat, 2010) and water temperature. Some authors found that otolith shape is related to life history traits (Vólpedo and Echeverria 2003; Mérigot et al. 2007) and biological and behavioural characteristics (like the type of swimming activity) (Aguirre and Lombarte 1999; De Vries et al. 2002; Lychakov and Rebane 2005). Mérigot et al.

(2007) have shown, using otolith morphometrics, that, for the common sole *Solea solea*, there are significant differences between sites belonging to the same type (within lagoons or within marine sites). They hypothesised that these differences could either be due to particular environmental conditions of each site and/or to specificities in metabolic and physiological conditions of the fish influencing otolith growth. It has been shown by several authors that environmental factors combined with physiological factors (like growth rate) are most influential in terms of otolith shape (Smith 1992; Campana and Casselman 1993; Friedland and Reddin 1994; De Vries et al. 2002). Otolith shape differences may also be due to biotic interactions, such as inter- and intra-specific competition for space given the highly territorial nature freshwater species (Keith and Lord *in press*), or for food or reproduction. These interactions have an effect on the fish's stress and therefore on their metabolism, which in turn influence the otolith growth and, hence its shape (Allemand et al. 2007). In rivers with different characteristics, fish may exhibit different types of behaviour in their adapting to these insular aquatic environments that are subject to extreme climatic and hydrological variations (Lord 2009). The substrate could also have an effect on the otolith shape (Vólpedo and Echeverria 2003) and it could especially affect *Sicyopterus* species, which feed on diatoms and

algae, scraping them off the hard substrate (Lord and Keith 2008).

Although environmental and biotic factors are probably the most significant (Campana and Casselman 1993), the differences evidenced in the otolith shape could also be linked to genetic effects (L'Abbé-Lund and Jensen 1993). Vignon and Morat (2010), working on the non-indigenous coral reef snapper (*Lutjanus kashmira*) showed that while the environment induces an overall change in otolith shape, genetic factors affect the otolith shape at a more local scale. Several authors have shown that the analysis of otolith morphology is useful for identifying fish stocks (Campana and Casselman 1993; Colman 1995; Reddin and Friedland 1999; Begg and Brown 2000; Bolles and Begg 2000; Tuset et al. 2003a, b; Pothin et al. 2006; Tracey et al. 2006), but, stock definition based on otolith shape depends on the environmental conditions encountered during the life history (Tuset et al. 2006). Moreover, life history during ontogeny may also affect the otolith shape (Anken et al. 1998). Life in different environments for specimens of the same species affects otolith shape (Morat et al. 2008), enabling the discrimination of different ecotypes.

Even with the different explanations put forward by the different authors to explain differences in otolith shape between and within species, it still remains unclear why we find, between western and central locations in Vanuatu, and for both species *S. lagocephalus* and *S. aiensis*, significant differences in otolith shape, as these locations belong to similar types of rivers, i.e., short and well oxygenated rivers on a volcanic substrate. It also still remains unclear why we find similarities between specimens caught in New Caledonia and on the western coast of Vanuatu knowing that the Caledonian river is on ultramafic substrate (nickel rich substrate of continental origin) while the Vanuatu island of Santo is of volcanic origin and the water conductivity is half that found in Vanuatu (Table 1). The very different geological origins mean that the substrates in New Caledonia and Vanuatu are very dissimilar (Lord 2009). Similarities found have thus to be explained differently.

The most probable explanation for the similarities found between New Caledonia and Western Santo, on the one hand, and Eastern Santo and Maewo (central locations), on the other, could be due to larval dispersal specificities and recruitment. These three *Sicyopterus* species are amphidromous. This means

that larvae undergo a migration towards the sea after hatching, where they spend a few months during which larval development takes place (McDowall 2007). In the Vanuatu/New Caledonia region, *S. lagocephalus* has a mean pelagic larval life of 130 days (Lord et al. 2010). This marine phase is the most important period of the life cycle regulating its dispersal and its ability to recruit (Keith 2003) and early life history at sea influences larval growth and metabolism (Anken et al. 1998). As a result, specimens caught in New Caledonia and on Vanuatu's West coast may have experienced similar early life histories, different from that of individuals caught in Vanuatu "central" locations. There may be different larval pools, due to current systems in the south Pacific. One would be located on the Western border of Vanuatu, colonising both western Vanuatu locations and New Caledonia using the south Vanuatu and the north and south Caledonian jets (Cillauren et al. 2001). It has indeed been shown that there is strong population connectivity between New Caledonia and Vanuatu due to ocean currents (Tremblay et al. 2008; Hamilton et al. 2010). The other larval pool would be confined to the "central" location of Vanuatu, representing a sort of internal "sea", at the centre of the archipelago, less penetrable by main ocean currents (Cillauren et al. 2001).

Nevertheless, further research is needed to quantify the role of genetic effects in comparison to environmental and biotic effects and to explain the discrimination observed in our results and the role of this region's currents.

Conclusion

The use of the otolith external outline has shown the importance of studying otolith morphology for discriminating *Sicyopterus* species and local populations. It seems that otolith morphometrics may be a useful method for differentiating between stocks on a phenotypic basis rather than on a genetic basis (Pothin et al. 2006), but in our case this has to be verified. Further studies are needed, such as precise measurements of environmental factors of the different rivers studied in combination with studies on the connectivity between populations using genetics (Lord 2009), marine current studies and the analysis of geochemical signatures in the otoliths. The differ-

ences and similarities observed have to be investigated in the light of the complexity of these species' life cycle, and especially the larval marine phase. Additionally, the behaviour of the fish (Lord 2009) and the study of the community organisation in relation to river particularities and natural disturbances (Monti and Legendre 2009) would also be interesting to understand the role of metabolism and physiological condition in otolith shape determinism. In any case, these first results on *Sicyopterus* otolith shape are promising, both for determining population structure, and for identifying the role of the environment on shaping the otolith.

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