Vol. 674: 1–13, 2021
https://doi.org/10.3354/meps13839

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published September 16

FEATURE ARTICLE

Historic dispersal barriers determine genetic structure and connectivity in a supratidal sandy-beach brooder

Karien Bezuidenhout[1,*], Ronel Nel[1], David S. Schoeman[1,2], Lorenz Hauser[1,3]

[1]Zoology Department, Nelson Mandela University, Port Elizabeth 6031, South Africa
[2]Global-Change Ecology Research Group, School of Science, Technology and Engineering, University of the Sunshine Coast, 4556 Sippy Downs, QLD, Australia
[3]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, USA

ABSTRACT: The supralittoral zones of sandy beaches are particularly vulnerable to coastal development and other activities that cause localised habitat destruction. Supratidal species, such as peracarid crustaceans, which lack a pelagic larval phase and tend to avoid direct contact with the swash, are therefore expected to be distributed as isolated populations, with implications for their long-term survival. Genetic population structure of one such species, the pill bug *Tylos capensis*, was investigated based on mitochondrial cytochrome *c* oxidase subunit I (COI) haplotype sequences, to assess demographic history and regional population connectivity in the presence of potential dispersal barriers (an estuary, coastal cliffs) along the Indian Ocean coast of South Africa. Here, pill bugs demonstrated strong and significant genetic structure at the regional scale, with 3 distinct clades across the species' geographic distribution. At a localised spatial scale, coastal cliffs intersecting the high shore appeared to be a strong barrier to gene flow between adjacent populations, while a permanently open estuary did not limit gene flow. Estimates of historic gene flow and patterns of COI differentiation coincided with greater habitat continuity during the Pleistocene glaciations at sea levels between −75 and −120 m, when the African south coast was probably dominated by sandy beaches. While gene flow among low-dispersing pill bug populations is unlikely to benefit from a network of closely spaced coastal protected areas, the isolated nature of this species, coupled with the cryptic diversity inherent in this taxon, emphasizes the need for their protection. The importance of protecting the intact littoral active zone of beaches is highlighted.

Sandy beach pill bugs (*Tylos capensis*) are prone to population isolation, which will impact their long-term survival without adequate protection.

Photo: Karien Bezuidenhout

KEY WORDS: Brooder · Dispersal barrier · Genetic connectivity · Sandy beach · Supratidal habitat

1. INTRODUCTION

Open coastlines are naturally patchy, heterogeneous habitats punctuated by rocky shores, mixed shores, wave-cut platforms, estuaries, and sea cliffs (e.g. Harris et al. 2011), in addition to man-made interruptions. Beaches and intertidal habitats are therefore generally disconnected and discontinuous

*Corresponding author: karien@sstafrica.org.za

Publisher: Inter-Research · www.int-res.com

at regional scales. Beach habitats show further variability, as sediment properties, wave action, and tidal amplitude vary at local and regional spatial scales to form different beach morphodynamic types (McLachlan et al. 2018). Locally, tidal elevation and water-table depth contribute to across-shore habitat variability, typically resulting in 3 across-shore zones (sublittoral, midlittoral, supralittoral), each with different microhabitat properties (Brown & McLachlan 2006). Beach invertebrate populations are distributed across these spatially heterogeneous seascapes, interspersed among regions of unsuitable habitats.

The supralittoral (or supratidal) is arguably the harshest of the tidal zones on exposed sandy beaches (Brown & McLachlan 2006). The supralittoral zone is positioned between the drift line and primary dunes. Aerial exposure, low levels of sand moisture, and extreme variations in air temperature render the supralittoral habitable only to species well adapted to deal with these conditions (Brown & McLachlan 2006). As such, the supratidal is dominated by crustaceans including ocypodid crabs, talitrid amphipods, and oniscid isopods, as well as several insect taxa (Brown & McLachlan 2006). A large proportion of these species is ovoviviparous (Defeo & Gómez 2005), exhibiting direct development or brooding. These species are important processors of beach wrack (e.g. Lowman et al. 2019), and so perform a crucial ecosystem function (Coupland et al. 2007). Despite their consistent ecological function, many species have a high degree of cryptic diversity and deep divergences among regional clades, e.g. oniscid ispods (Hurtado et al. 2013, 2014, Niikura et al. 2015) and talitrid amphipods (Pavesi & Ketmaier 2013, Baldanzi et al. 2016). The supratidal zone, although being physically extreme, therefore harbours unique and often cryptic faunal diversity of ecological importance.

The beach supratidal zone is also particularly vulnerable to anthropogenically induced habitat loss and fragmentation (Ketmaier et al. 2003, Dugan et al. 2008). Impacts are often in the form of coastal developments (e.g. seawalls, buildings) that are typically concentrated on the high-shore and in the supratidal (Defeo et al. 2009, Pilkey & Cooper 2014). In addition, beach grooming, trampling, and vehicle use on the beach further increase the loss of supralittoral habitat (Defeo et al. 2009, Dugan et al. 2010). The lack of a pelagic larval phase in many supratidal species (Defeo & Gómez 2005, Brown & McLachlan 2006) may limit connectivity among populations, thus decreasing rates of (re)colonization (Pechenik 1999) of beaches and increasing the risk of inbreeding and local extirpation (Hubbard et al. 2014).

Anecdotal as well as empirical evidence already indicates that supralittoral fauna are impacted by habitat degradation and subjected to habitat fragmentation. For example, Brown (2000) noted the disappearance of *Tylos granulatus* from several beaches along the South African west coast, while Deidun et al. (2011) reported a severe contraction in the distribution of *T. europaeus* and *T. sardous* (= *T. ponticus*) populations on Maltese beaches. Hubbard et al. (2014) likewise demonstrated the extirpation of *T. punctatus* from 57% of beaches originally occupied in southern California, USA, due to habitat loss, and the loss of another isopod, *Alloniscus perconvexus*, from 64% of these beaches. Ketmaier et al. (2005) showed a reduction in genetic variation in *Talitrus saltator* on beaches subject to human disturbance. Finally, urbanization and associated impacts have also been implicated in the decline of supratidal tiger beetles (Nagano 1980 in Hubbard et al. 2014). With threats such as development, sea-level rise, and erosion projected to increase (Defeo et al. 2009, Pilkey & Cooper 2014), supralittoral populations and species are likely to become increasingly vulnerable in the future.

Pill bugs of the genus *Tylos* are considered good indicators of habitat quality (Gonçalves et al. 2005, Deidun et al. 2011) and could therefore also be indicative of the effects of habitat fragmentation. The genus is globally distributed, although most representatives inhabit tropical and subtropical regions (Brown & Odendaal 1994). Two species inhabit temperate South Africa: *T. capensis*, an endemic to the south coast, and *T. granulatus,* which is limited to the west coast of South Africa and Namibia (Kensley 1974). *Tylos* spp. require beaches with a sufficient back (supratidal) beach, and most pill bugs actively avoid contact with the swash (Kensley 1974, Brown & Odendaal 1994). Juveniles, in particular, are occasionally trapped in the swash where they can survive submersion up to 24 h (Kensley 1974); floating is enabled by air pockets trapped between the pereiopods and pleopods (Menzies 1952, Kensley 1974), and pill bugs might be able to use algae or other allochthonous inputs on beaches as rafts to facilitate dispersal (Brown & Odendaal 1994). Nevertheless, colonization events appear to be rare (Brown & Odendaal 1994). Pill bugs therefore exhibit characteristics associated with an elevated extinction risk (see Roberts & Hawkins 1999 for criteria), including their limited dispersal capacity, limited geographic distribution, habitat specificity, patchy occurrence across their range, and high levels of sensitivity to human impact (Brown & Odendaal 1994, Hubbard et al. 2014).

In highly fragmented species such as *Tylos* spp., genetic results may be important in directing conservation efforts and management policy (von der Heyden et al. 2014). Genetic tools can be applied to assess population connectivity, and so also inform decisions regarding the location of, and distances between, marine reserves necessary for the effective protection of species (e.g. von der Heyden 2009, Wright et al. 2015). Genetic studies may assist in prioritizing species or populations for conservation by identifying cryptic biodiversity (e.g. Hurtado et al. 2013) and diversity hotspots (e.g. von der Heyden 2009).

We used the mitochondrial marker cytochrome *c* oxidase subunit I (COI) to assess genetic structure in the pill bug *T. capensis*, within a naturally patchy seascape along the south coast of South Africa. Two hypotheses were tested to investigate population differentiation in *T. capensis* at a broad spatial scale along its range, and to assess the role of putative dispersal barriers at a small spatial scale in Algoa Bay. First, congruent to the findings for other oniscid isopods (e.g. Niikura et al. 2015, Mbongwa et al. 2019), we hypothesised that *T. capensis* is genetically differentiated across its range, along a distance of ca. 600 km on the southwest Indian Ocean seaboard of South Africa, as expected from very low dispersal rates and connectivity. Second, the potential roles of natural coastal features as dispersal barriers were investigated in Algoa Bay, which is characterised by several sandy beaches separated by estuaries, a sea cliff, and stretches of rocky shore. Investigating population structure within a single bay, where study locations are subjected to similar past and contemporary environmental conditions, may help in identifying barriers to dispersal and drivers of genetic structure. Conducting such studies at small spatial scales could assist in elucidating scales of connectivity and is hence crucial for conservation programmes. We hypothesised that a permanently open estuary and a sea cliff represent dispersal barriers between adjacent sampling locations, resulting in significant genetic differentiation in pill bugs at small (ca. 60 km) spatial scales. The implications for conservation and population persistence of *Tylos* spp., and sandy beach supralittoral communities in general, were also highlighted.

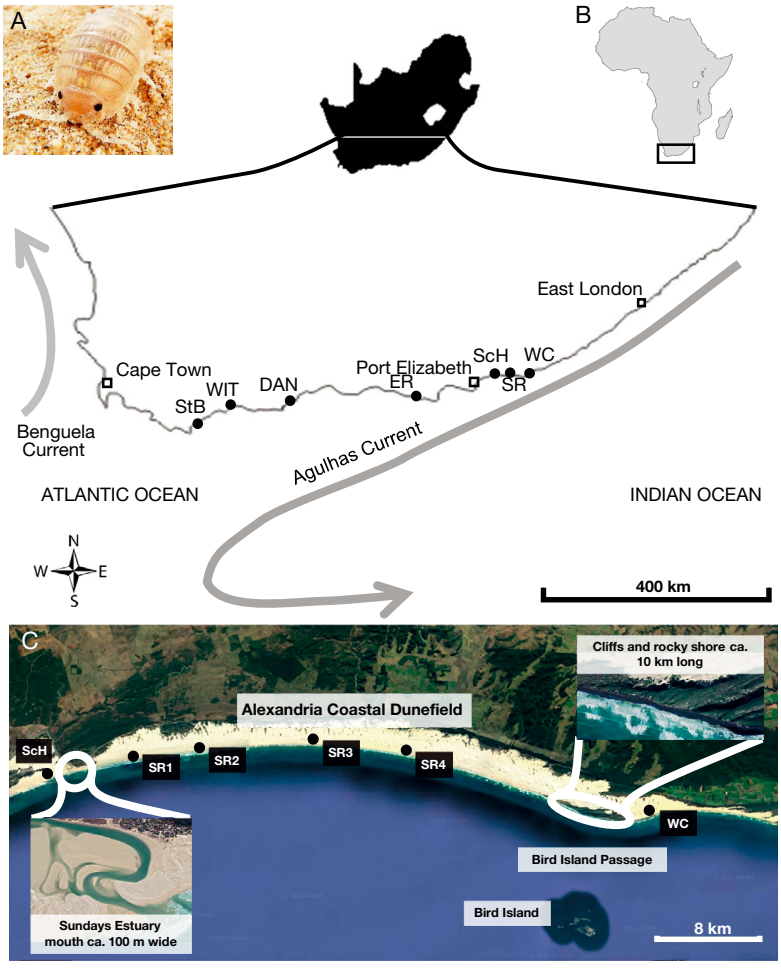

Fig. 1. Sample locations of (A) *Tylos capensis* along (B) the south coast of South Africa. (C) Details of 3 beaches (ScH, SR, WC, representing 6 sample locations) sampled within Algoa Bay, with the Sundays River Estuary and cliffs separating Sundays River Beach and Woody Cape. Sample locations: Struisbaai (StB), Witsand (WIT), Dana Bay (DAN), Eersterivier (ER), Schelmhoek (ScH), Sundays River Beach (SR, 4 locations), Woody Cape (WC) (*Tylos capensis* photo: K Bezuidenhout; Algoa Bay image: Google Earth; 33° 45′ 05″ S, 25° 58′ 26″ E, eye altitude 46 km; accessed on 16 July 2021)

2. MATERIALS AND METHODS

2.1. Sample collection and processing

To assess the regional pattern of population structure, *Tylos capensis* were collected from 10 sampling locations on 7 beaches along the south coast of South Africa from Struisbaai in the west to Woody Cape towards the east (Fig. 1A,B). Beaches further east of

Woody Cape to East London were visited but no pill bugs were found. The south coast is characterised by log-spiral bays, dominated by sandy beaches of intermediate to dissipative-intermediate morphodynamic type (Harris et al. 2011). Beaches are separated by rocky shores and headlands, and several estuaries. The longest continuous stretch of rocky coastline (~80 km), Tsitsikamma, is situated along the south coast between Dana Bay and Eersterivier (Fig. 1).

Small-scale patterns of population structure and barriers to gene flow were investigated at 6 of the 10 sampling locations located in Algoa Bay (Fig. 1C). Schelmhoek Dunefield is situated west of the permanently open Sundays River Estuary. The estuarine mouth, which separates Schelmhoek Beach from Sundays River Beach to the east, is approximately 100 m wide. Sundays River Beach is 40 km long and backed by the Alexandria Coastal Dunefield (ACD). The ACD, comprising transverse dunes rising to a height of approximately 150 m, is the largest coastal dune system in South Africa (Illenberger & Rust 1988). Net dune movement takes place in a northeasterly direction, as determined by the direction of the prevailing winds (McLachlan 1991). The area is near-pristine, with no development within the littoral active zone (the area from the surf zone to the foredunes), which allows for unhindered dune movement. The 50 km long dunefield (Illenberger & Rust 1988) backs both the Sundays River Beach and Woody Cape Beach, although the 2 beaches are interrupted by a ~10 km continuous, steep calcarenite sea cliff, on the high-water mark (Fig. 1C). Four locations (SR1–4) were sampled on the Sundays River Beach (Fig. 1C).

Pill bugs were collected by means of 30–40 pitfall traps (1 l containers, baited with peanut butter) at each location, set at dusk in the supratidal and retrieved the following morning. Specimens were preserved in absolute (99%) ethanol until further processing. DNA was then extracted from isopod legs, to avoid possible contamination by gut contents, using a Gentra® Puregene® Tissue Kit (Qiagen), following the manufacturer's protocol for preserved tissues. A fragment of ca. 710 bp of COI was amplified with the universal primers (LCO1490 and HC02198) of Folmer et al. (1994) in 20 µl reactions, using Kapa *Taq* 2× ReadyMix DNA Polymerase (Kapa Biosystems), with 0.25 µM of each primer. Thermal cycling was performed at 95°C for 2 min (initial denaturation), 35 cycles of 95°C for 1 min, annealing at 49.4°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min (final extension). Attempts to amplify and sequence additional markers, including 16S, cytochrome *b*, 18S (Giribet et al. 1996, Merritt et al. 1998), and ND2 (using a novel primer) failed, and subsequent analyses were limited to COI.

Amplicons were visualised with ethidium bromide on 0.7% agarose gels prepared with Tris-acetate-EDTA buffer. Sequencing was subsequently performed by Inqaba Biotechnologies (South Africa), and the High Throughput Genomic Centre (University of Washington, USA). Samples (n = 39) of COI were sequenced in both directions to determine the suitability of the primers and to detect inconsistencies between forward and reverse sequences. We found no discrepancies between forward and reverse sequences, and the remaining samples were therefore sequenced in one direction (LCO1490) only. Sequences were aligned and edited in Geneious 6.1.8 (Biomatters, USA) and checked to verify the absence of double peaks indicating heteroplasmy (GenBank accession numbers: MZ540108–MZ540140). Codons were translated to amino acids in Geneious Ver. 6.1.8 to determine if nucleotide substitutions were synonymous or not, and to inspect sequences for the presence of in-frame stop codons that could indicate nuclear mitochondrial pseudogenes (NUMTs; Song et al. 2008); no evidence of NUMTs and heteroplasmy was found.

2.2. Data analyses

2.2.1. Genetic diversity and population structure

Unbiased indices of molecular diversity were estimated for all sample locations in DnaSP 5.10 (Librado & Rozas 2009) and Arlequin 3.5 (Excoffier & Lischer 2010). Haplotype networks were constructed in Haploviewer (www.cibiv.at/~greg/haploviewer, (Salzburger et al. 2011), based on a maximum likelihood phylogenetic tree, constructed in Phylip 3.69 (Felsenstein 1989). MEGA 6 (Tamura et al. 2013) was used to determine the most appropriate nucleotide substitution model (i.e. the model that scored the lowest Bayesian information criterion value). Pairwise genetic distances, corrected for Tamura 3-parameter (Tamura 1992) nucleotide substitution, were subsequently determined among haplotypes.

Hierarchical population structure, comparing variance based on nucleotide differences among all sample locations ('populations') (Φ_{ST}), among groups (Φ_{CT}), and among sample locations within groups (Φ_{SC}) was estimated in Arlequin (Excoffier & Lischer 2010) as an analysis of molecular variance (AMOVA, Excoffier et al. 1992). Groups were defined *a priori*

based on the clades identified in the haplotype network. An AMOVA was implemented with Tamura 3-parameter nucleotide substitution model (Tamura 1992) with 0.05 gamma correction. Significance was determined with 10 000 permutations (Excoffier et al. 1992). Pairwise Φ_{ST} values based on nucleotide differences between sequences were estimated for pill bugs among all study locations and tested for significant deviation from zero with 10 000 permutations. Differences in haplotype frequency among populations were assessed with an Exact test, with 100 000 Markov chain steps and 10 000 dememorization steps (Raymond & Rousset 1995), performed in Arlequin. Critical p-values for multiple pairwise tests were corrected to account for false discovery rates by applying the B-Y correction described by Benjamini & Yekutieli (2001) and Narum (2006). Pairwise genetic distances among individual pill bugs were determined in MEGA 6 (Tamura et al. 2013) based on the Tamura 3-parameter model.

2.2.2. Gene flow

Gene flow ($M = m/\mu$, mutation-scaled migration rates) and effective population size ($\theta = N_e\mu$, mutation-scaled effective population size) were estimated from Bayesian coalescence modelling in Migrate-N 3.4.6 (Beerli & Felsenstein 2001, Beerli 2009). N_em, the number of immigrants per generation independent of mutation rate, was calculated by multiplying θ and M estimated in Migrate-N. N_em estimated from coalescence approaches (such as those implemented in Migrate-N) is an average of immigration over the past N_e generations for mitochondrial markers (although biased towards estimates in more recent time; Beerli 2009) and as such reflects contemporary as well as historic gene flow.

Gene flow was estimated first for sample locations across the range of *T. capensis*, assuming a bidirectional stepwise migration model between study locations. Due to the constraints associated with estimating many parameters, adjacent study locations not significantly differentiated from each other (in terms of pairwise Φ_{ST} values) were pooled to form 5 assemblages (i.e. not the same groups as implemented in the AMOVA), viz. (1) Struisbaai; (2) Witsand; (3) Dana Bay and Eersterivier; (4) Schelmhoek and Sundays River Beach locations (SR 1 to SR 4); and (5) Woody Cape. Second, gene flow was estimated for pill bugs in Algoa Bay, across the 2 putative barriers and along the continuous beach, assuming a stepwise eastwards migration model. Study locations were again pooled when these were not differentiated significantly, for reasons stated above: (1) Schelmhoek; (2) SR 1 and 2; (3) SR 3 and 4; and (4) Woody Cape. Each run was performed using Bayesian inference, and consisted of 3 replicates of 2 000 000 steps each, with 10 000 burn-in steps. Analyses were implemented with Metropolis-Hastings sampling, exponential priors (which in initial test runs yielded better convergence of posterior distributions than did uniform priors), and 4 heated chains (i.e. 1.0, 1.5, 3.0, 1 000 000). Other migration models (e.g. bidirectional migration in Algoa Bay) were tested in Migrate-N, but these did not converge and were not pursued further.

2.2.3. Marker neutrality and population demographics

Departures from neutrality in *T. capensis* COI and signatures of past population expansion were examined by determining the D (Tajima 1989) and F_s (Fu 1997) statistics in Arlequin (with 1000 simulations), and R_2 (Ramos-Onsins & Rozas 2002) in DnaSP 5.10 (Librado & Rozas 2009). The demographic histories were further assessed by means of pairwise mismatch distributions under both the population and range-expansion models (Rogers & Harpending 1992, Rogers 1995) for 3 separate groups (i.e. south-western, south-eastern, Woody Cape). Harpending's raggedness index (Hri, Harpending 1994) and the sum of squared deviations (SSD), time since expansion (τ), initial and final population sizes (θ) were estimated in Arlequin (Excoffier & Lischer 2010), with 95% confidence intervals for 1000 bootstrapped iterations.

3. RESULTS

3.1. Genetic diversity and population structure

The final sequence assembly consisted of 581 bp and 227 individuals, which yielded 33 haplotypes with 34 substitutions and 32 segregating sites (S, Table 1). Haplotype (h) and nucleotide (π) diversity ranged from to 0.157 to 0.618, and from 0.0007 to 0.002, respectively (Table 1). Nucleotide substitutions were predominantly transitions and almost exclusively synonymous, with the exception of one non-synonymous substitution recorded from Struisbaai (Table 1). Woody Cape pill bugs had the lowest haplotype diversity, but average nucleotide diversity.

Table 1. Diversity indices for *Tylos capensis*, collected from 10 sites on 7 beaches: *H*: number of haplotypes; *h*: haplotype diversity; π: nucleotide diversity; *S*: number of segregating sites on sequences; Subst.: number of substitutions; *Ks*: number of synonymous substitutions; *Ka*: number of non-synonymous substitutions; T_S: number of transitions; T_V: number of transversions; Priv. subst.: number of private substitution sites; Priv. haps: private haplotypes. Site names abbreviated as in Fig. 1

	StB	WIT	DAN	ER	ScH	SR1	SR2	SR3	SR4	WC	Total	Mean ± SD
n	20	14	25	24	28	34	19	27	11	25	227	22.7 ± 6.83
H	5.7	2.8	4.8	3.8	5.8	5.8	4.7	5.8	4.5	2.9	32.9	4.8 ± 1.14
h	0.550	0.360	0.350	0.240	0.470	0.400	0.440	0.430	0.560	0.150	0.780	0.401 ± 0.1211
π	0.0014	0.0007	0.0007	0.0005	0.0016	0.0010	0.0013	0.0010	0.0020	0.0012	0.011	0.0011 ± 0.00046
S	4.8	1.9	3.8	2.9	5.8	4.9	5.7	5.8	6.4	7.7	31.9	4.9 ± 1.81
Subst.	4.8	1.9	3.8	2.9	5.8	4.9	5.7	5.8	6.4	7.7	33.9	4.9 ± 1.72
Ks	3.8	1.9	3.8	3	5.8	4.9	5.7	5.8	6.4	7.7	33	5.1 ± 1.85
Ka	1	0	0	0	0	0	0	0	0	0	1	0.1 ± 0.32
T_S	2.9	1.9	3.8	1.9	5.8	4.9	4.7	5.8	6.4	6.7	28.9	4.5 ± 1.79
T_V	1.9	0	0	1	0	0	1	0	0	1	5	0.5 ± 0.67
Priv. subst.	3.8	0.9	1	2.9	0	1	1.9	1	2.7	3.8	19.9	1.9 ± 1.34
Priv. haps.	3.8	0.9	2.9	2.9	0.0	1.9	1.9	1.0	2.7	1.0	19.9	1.9 ± 1.18

Patterns of population differentiation were dominated by a sharp west–east divide between Eersterivier and Schelmhoek. The haplotype network showed no shared haplotypes between the western (Struisbaai, Witsand, Dana Bay, Eersterivier) and eastern groups (Schelmhoek, SR 1–SR 4), which were separated by 10 substitutions. Woody Cape *Tylos* represented a third group (Fig. 2A). Only one haplotype was shared between Woody Cape and south-eastern pill bugs. Genetic distances between pairs of haplotypes ranged from 0.17 to 2.83% for Tamura 3-parameter correction (Table S1 in the Supplement at www.int-res.com/articles/suppl/m674p001_supp.pdf).

Strong, significant population structure was estimated for *T. capensis* across all study locations (AMOVA without group assignment: Φ_{ST} = 0.90, $p < 0.0001$). An AMOVA, using the same 3 groups identified in the haplotype network, again revealed strong, significant, population structure, based on nucleotide differences, across all study locations (Φ_{ST} = 0.93, $p < 0.0001$), as well as among the 3 groups (Φ_{CT} = 0.92, $p < 0.0001$), but showed much less, yet significant, variation among sites within groups (Φ_{SC} = 0.15, $p < 0.0001$). Pairwise population differentiation based on nucleotide differences (Φ_{ST}) revealed strong significant differentiation among most study locations, except among Sundays River Beach sites (SR1–SR4) and Schelmhoek, and between Dana Bay and Eersterivier (Table 2). The Exact test yielded results similar to that of the pairwise Φ_{ST} estimates (Table 2; global p-value < 0.0001), strongly rejecting the null hypothesis of equal haplotype frequencies.

3.2. Gene flow

The estimated number of effective migrants (N_em) was generally low between groups; from Witsand to neighbouring groups, dispersal was relatively high, while dispersal into Witsand was very low (Fig. 2B; Table S2). Dispersal rates in Algoa Bay were high between Schelmhoek and SR1 and 2, and between SR 1 and 2, and SR 3 and 4 (Fig. 2C; Table S2). Mutation-scaled effective population sizes (θ) for the 5 groups were low, although estimates for the populations of Struisbaai and Schelmhoek/Sundays River were an order of magnitude larger than those of the other study locations (Fig. 2B,C; Table S2). Corresponding to the geographic pattern of the haplotype network, migration between the 2 main regions (south-west and south-east) was estimated to be zero.

3.3. Marker neutrality and population demographics

Tests for marker neutrality (Tajima's *D*, F_S) for the 3 groups were significantly negative in most instances, thus rejecting the null hypothesis of neutrality (i.e. no selection and constant population size; Fig. 3; Table S3). F_S estimated for the Woody Cape population (group), however, did not reject neutrality (Fig. 3, Table S3). At the level of individual populations (beaches), estimates of Tajima's *D* and F_S were negative in all cases, supportive of purifying selection or recent expansion (Table S3). Tajima's *D* was significant for Dana Bay, SR 2–

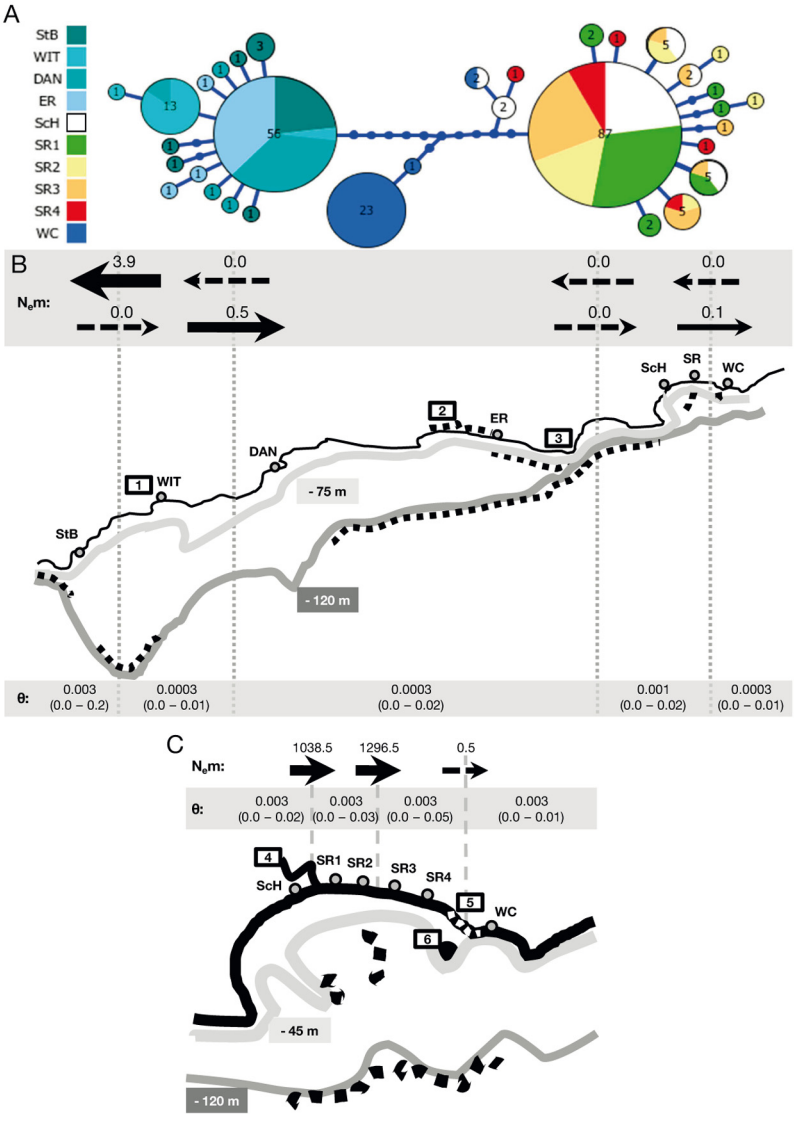

Fig. 2. Haplotype network and migration rates for *Tylos capensis* along the south coast. (A) Haplotype network. Each circle represents a unique haplotype, and numbers in, and size of, circles indicate the frequency of that haplotype. Each branch indicates 1 mutational step; small, solid circles on branches denote unsampled mutations. Sampling locations are shown in different colours and abbreviated as in Fig. 1. (B,C) Estimates of gene flow among, and effective population size for (B) 5 pooled sampling locations along the South African south coast, and (C) 4 pooled sampling locations in Algoa Bay, superimposed on a schematic of palaeocoastlines (individual sampling locations also shown). Grey dashed lines indicate the boundaries of pooled populations implemented in Migrate-N. Estimates of the modal number of effective migrants (N_em) between adjacent locations are indicated with black arrows, where the arrows represent the relative magnitude and direction of gene flow; dashed arrows indicate a modal N_em = 0.0 (see Table S2 for confidence intervals). Population sizes are given as modal θ-values, with 2.5–97.5 percentiles, for each group of sampling locations. Palaeo-sea levels at –75 m/–45 m (interglacial period) and –120 m (Last Glacial Maximum) are visualised in light and dark grey lines, respectively, as per Dingle & Rogers (1972) and Toms et al. (2014); black dashed lines indicate predominantly rocky shore areas. Coastal features: 1: Breede River adjacent to Witsand; 2: Tsitsikamma rocky shore; 3: Plettenberg Bay Portal (sensu Compton et al. 2011); 4: Sundays River Estuary; 5: sea cliff at 'The Krans' (white dashed line); 6: Bird Island

SR 4, and Woody Cape, while marker neutrality was similarly rejected for Struisbaai, Dana Bay, Eersterivier, and Sundays River 1 and 3, based on Fu's F_S (Table S3). Estimates of R_2 were significant p < 0.05) in all cases. Mismatch distributions and estimates for Hri and SSD, for the 3 groups, did not reject the population or the range-expansion models (p > 0.05 in all cases; Fig. 3, Table S4). Similarly, neither the demographic expansion nor the range-expansion model was rejected for individual populations, except Schelmhoek and SR 2 (demographic expansion model rejected: SSD 0.3, p < 0.05, Table S3).

4. DISCUSSION

This study aimed to assess genetic structure and population connectivity in the high-shore crustacean *Tylos capensis* across (1) its range along ~600 km of the South African south coast and (2) at a small spatial scale (~60 km) to assess the roles of a permanently open estuary (Sundays River) and a sea cliff (Woody Cape) as potential barriers to gene flow. The results broadly supported the expectations for this supralittoral, direct developer. First, pill bugs generally displayed strong population structure across their range, showing 3 groups with distinct regional distributions (Fig. 2A). Second, at a small spatial scale, the virtual absence of pairwise (Φ_{ST}) genetic differentiation, coupled with the large number of migrants (N_em) supported the notion of high population connectivity along the continuous Sundays River Beach (Fig. 2C). Patterns of differentiation also indicated that the sea cliff between Woody Cape and Sunday River Beach acted as a barrier to gene flow, whereas the Sundays River Estuary did not. The results generally support the hypothesis that pill bugs are distributed predomi-

Table 2. Pairwise Φ_{ST} values (below the diagonal), and significance of exact tests (above the diagonal) for COI sequences of *Tylos capensis* collected from 10 locations. *p < 0.012 (critical p-value after B-Y correction for multiple comparisons); **p < 0.0001; NS: not-significant. Site names abbreviated as in Fig. 1

	StB	WIT	DAN	ER	ScH	SR1	SR2	SR3	SR4	WC
StB		**	NS	NS	**	**	**	**	**	**
WIT	0.53**		**	**	**	**	**	**	**	**
DAN	0.06*	0.60**		NS	**	**	**	**	**	**
ER	0.06*	0.64**	0.02		**	**	**	**	**	**
ScH	0.92**	0.92**	0.94**	0.94**		NS	NS	NS	NS	**
SR1	0.94**	0.95**	0.96**	0.96**	0.04		NS	NS	NS	**
SR2	0.93**	0.94**	0.95**	0.95**	0.00	0.02		NS	NS	**
SR3	0.94**	0.95**	0.96**	0.96**	0.04	0.02	0.00		NS	**
SR4	0.91**	0.93**	0.94**	0.94**	−0.02	0.04	−0.01	0.00		**
WC	0.89**	0.93**	0.93**	0.92**	0.89**	0.92**	0.91**	0.92**	0.89**	

nantly as isolated populations across their range, particularly where populations are separated by dispersal barriers, but also indicate the effects of demographic history.

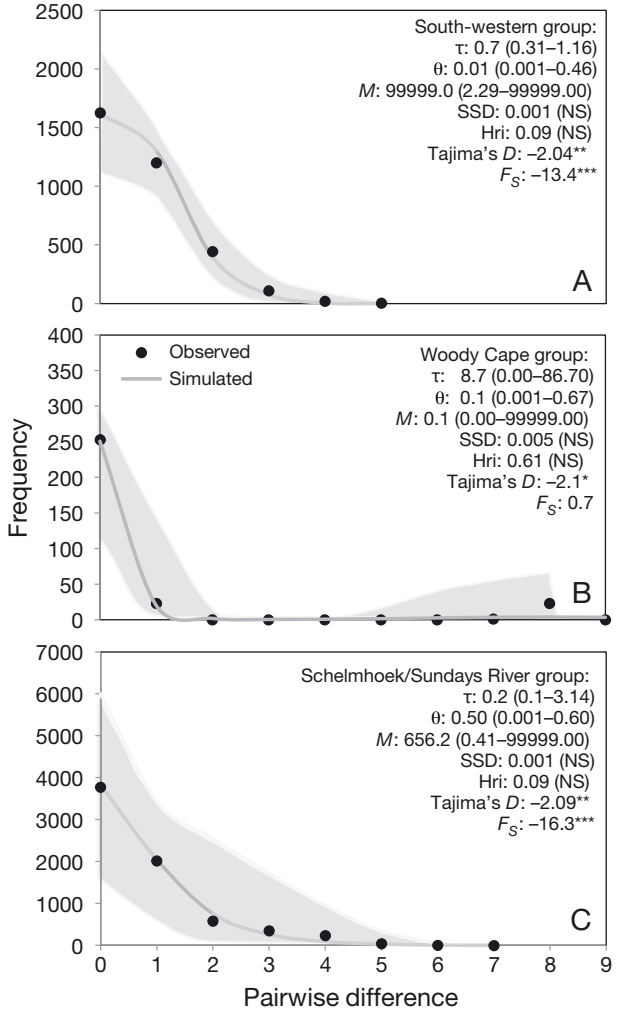

Fig. 3. Mismatch distributions for *Tylos capensis*, according to the spatial-expansion models for (A) the south-western, (B) the Woody Cape, and (C) the Schelmhoek/Sundays River (south-eastern) groups. Solid black circles indicate the observed trend, and dark grey lines show the simulated trends. Light grey-shaded areas delineate the 95% confidence interval. Note the different scales on the *y*-axes. Mutation-scaled effective population size (θ), mutation-scaled immigration rates (*M*), with 95% confidence intervals in parentheses, and estimates for the sum of squared deviation (SSD) and Harpending's raggedness index (Hri) are also shown for each clade. Estimates for population and range expansion models were very similar in all cases. *p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant

4.1. Genetic differentiation at a regional scale

Supralittoral communities are thought to harbour considerable cryptic diversity (Hurtado et al. 2013, 2014, Greenan et al. 2018), so the distinct genetic and spatial separation of groups demonstrated here for *T. capensis* might suggest the existence of unique species or subspecies. However, while genetic distances among the haplotypes of *T. capensis* (0.17–2.83%) were more pronounced than those among populations of *T. punctatus* (Clade A in Hurtado et al. 2013), they were well below the pairwise COI differences reported between known species of *Tylos* (11.5–42%, Hurtado et al. 2014; see also Mbongwa et al. 2019) and between species of *Ligia*, another oniscid isopod (13.2–26.7%; Markow & Pfeiler 2010). Furthermore, only one (private) nucleotide substitution was non-synonymous in *T. capensis*. Therefore, *T. capensis* occupying the South African south coast should be considered a single species based on the COI marker.

There was, nonetheless, strong genetic differentiation among the 3 groups (Φ_{CT}), and among (Φ_{ST}) and between (pairwise Φ_{ST}) most populations of *T. capensis,* with only some exceptions. COI differentiation in

T. capensis was comparable to that of other supratidal direct developers, including isopods (Niikura et al. 2015, Mattos et al. 2019, Mbongwa et al. 2019) and talitrid amphipods from beaches lacking large wrack inputs (De Matthaeis et al. 2000, Pavesi & Ketmaier 2013). Strong, significant estimates of population structure were also demonstrated for peracarids occupying lower tidal levels on the beach with ready access to water (e.g. Varela & Haye 2012, Takada et al. 2018), although there are exceptions (e.g. Tourinho et al. 2016). The generally strong genetic differentiation and low gene flow (N_em) in *T. capensis* suggest low connectivity, which is consistent with expectations for species with very low vagility living in fragmented habitats.

4.2. Genetic differentiation and dispersal barriers at a small spatial scale

Patterns of gene flow within Algoa Bay indicated that some coastal features could represent dispersal barriers to pill bugs. Significant population structure and low N_em values between Sundays River Beach and Woody Cape coincided with the presence of the sea cliffs separating these 2 beaches. In contrast, the permanently open estuary appeared not to have limited gene flow, as indicated by non-significant genetic differentiation and large N_em values between Schelmhoek and Sundays River Beach. These 2 coastal features differ in their spatial scales. The estuary mouth is approximately 100 m wide, perhaps allowing short stochastic dispersal events via the swash, while the ~10 km long coastal cliffs are more extensive. Spatial extent might hence account for differences in the effectiveness of the features as dispersal barriers. The large N_em values estimated along the continuous Sundays River Beach are supported by previous observations of *T. granulatus* that colonized a newly formed sandy berm across the Orange River mouth (west coast, South Africa) from the adjacent, continuous, sandy beach, over a period of 2 yr (Brown & Odendaal 1994). Elsewhere, Odendaal et al. (1999) estimated a theoretical maximum travel distance, alongshore, of 936 m per 2 h activity window for *T. granulatus* without feeding (or 80 m over 2 h with frequent stops). The results presented for *T. capensis* therefore provide tentative support that migration and hence gene flow in pill bugs is influenced by the degree of contemporary habitat continuity.

The sea cliffs and estuary also differ in their temporal persistence. Aerial photographic evidence suggests the Sundays River Estuary has been permanently open for at least the past 80 yr (S. Taljaard unpubl. data), but past climatic variability, including a warmer and drier period relative to the present (the Holocene Maximum; Partridge et al. 1999), could have resulted in periodic mouth closures. Homogeneous genetic structure across the Sundays Estuary might therefore reflect historic connectivity, because very recent divergence or range and population expansion events could produce the same lack of population differentiation (Whitlock & McCauley 1999, Hart & Marko 2010). Mismatch analyses and a star-shaped phylogeny confirmed that range and/or population expansion events took place (Slatkin & Hudson 1991) in *T. capensis*. In contrast, the sea cliffs at Woody Cape probably formed with rising sea levels during the Pleistocene. The earlier relative time since expansion (τ) estimated for pill bugs from Woody Cape Beach is consistent with an early range expansion into Woody Cape followed by a vicariance event (formation of the sea cliff) which separated this population relatively early from that of Schelmhoek/Sundays River (unfortunately, time of divergence between the Woody Cape and Sundays River Beach pill bugs is unknown). Although infrequent stochastic water dispersal and subsequent gene flow from Schelmhoek/Sundays River Beach to Woody Cape Beach is possible, the shared haplotype between these study locations and very low estimated N_em likely reflects ancestral polymorphism. Low rates of gene flow into and from Woody Cape is therefore likely due to an ancient divergence and is presently maintained by the sea cliff.

4.3. Other drivers of genetic differentiation

Although not explicitly tested here, palaeocoastlines could explain the observed genetic structure in pill bugs at different geographic scales. The palaeo-sea level along the southern African coast retreated after the Last Glacial Maximum (at sea levels between −120 and −45 m), exposing more, largely continuous, sandy beach habitat particularly over the Agulhas Bank (Van Andel 1989, Toms et al. 2014). Dispersal facilitated by the increased habitat connectivity among Dana Bay and Eersterivier (as historic population connectivity) or the recent divergence following a range expansion from a single ancestral population during this time, might explain the lack of genetic differentiation between these 2 sites. Unfortunately, these scenarios could not be distinguished because divergence times are not known. The rapid narrowing of the continental shelf in the vicinity of

Cape St Francis, i.e. the Plettenberg Bay Portal (sensu Compton 2011; Fig. 2B), was characterised by rocky substrate at most palaeo-sea levels (Dingle & Rogers 1972). Estimates of effective dispersal along the south coast, as well as the spatial distribution of haplotypes, provides evidence for Plettenberg Bay Portal as an historic barrier to dispersal in *T. capensis.*

Genetic structure in *T. capensis* is likely also driven by small effective population sizes, further maintained by low rates of migration. Female pill bug population sizes, here estimated as θ, were generally small. Coupled with N_em values estimated here as 0.0–3.9 immigrants per generation, it is conceivable that at least some populations of *T. capensis* would be subjected to inbreeding and genetic drift, as expected for small populations (see Lowe & Allendorf 2010). Furthermore, pill bug sex ratios are male-biased (Gonçalves et al. 2005, K. Bezuidenhout pers. obs.), which may also reduce female effective population size. Population differentiation in *T. capensis* may therefore be further enhanced and maintained by small female population sizes, in addition to low vagility.

Other processes not considered here may contribute to the observed patterns of COI differentiation in *T. capensis*. These include the possibility of male-biased dispersal, which cannot be detected with mitochondrial DNA (Hübner et al. 2015), or sex-based survival of migrants (Baker & Rao 2004). Finally, *Wolbachia*, a maternally inherited bacterium, is widespread among arthropods and can have a marked effect on host population genetic structure (Hurst & Jiggins 2005). Nonetheless, geographic patterns of differentiation in *T. capensis* are congruent with historic sea levels and low concomitant gene flow, and broadly resemble the findings of previous studies (e.g. Hurtado et al. 2013), giving credence to the interpretation here.

4.4. Implications and concluding remarks

The genetic patterns in *T. capensis*, i.e. the small effective female population sizes and low connectivity, resemble those of populations at risk of habitat fragmentation and extinction (Frankham 1996, Keyghobadi 2007). As such, our results support previous assertions of high extirpation vulnerability of pill bugs because of their limited colonization capacity in fragmented habitats (Brown 2000, Hubbard et al. 2014). Large-scale coastal development along the South African coast is still reasonably limited (Harris et al. 2014) and confined to a few coastal nodes, but erosion is a serious risk (Bird 2000). Other impacts, such as artificial light, which has been shown to affect animal behaviour and physiology, also extends beyond the boundaries of coastal cities (Gaston et al. 2015), with potentially severe consequences for the survival of sandy beach supratidal fauna (Luarte et al. 2016), including *T. capensis.* Furthermore, sandy beaches represent several different types of habitats, commonly described as different morphodynamic states (McLachlan et al. 2018). Not all beach types are suitable to all beach species, with some species showing greater habitat specificity than others (Brown & McLachlan 2006, Defeo & McLachlan 2011). Consequently, beach habitats are likely more fragmented than typically perceived, particularly at contemporary timescales.

Marine reserves of sufficient size, spaced at appropriate distances to facilitate stepwise dispersal, are recognized as an effective means to protect marine fauna (Wright et al. 2015). Considering, however, the low dispersal ability of *T. capensis* and potentially of other supratidal taxa, it is unlikely that these species would benefit from a network of closely spaced reserves to facilitate movement among beaches, at least on ecological time scales (although this might benefit connectivity in rafting talitrid amphipods; Pavesi & Ketmaier 2013). Rather, the full protection of a number of extensive sandy beaches with intact littoral active zones to allow the natural retreat of the beach with sea-level rise, which also accommodate large populations of supralittoral invertebrates, would be more efficient than a series of many smaller or partially protected beaches. Adequate protection of species such as *T. capensis* should include at least a representative of each of the dominant groups (von der Heyden et al. 2014).

In conclusion, the pill bug *T. capensis* shows strong population differentiation across its range, as was shown for congeners elsewhere as well as for other supratidal taxa. The patterns of gene flow and population structure estimated here were consistent with reconstructed palaeo-shorelines and beach habitat connectivity, suggesting that dispersal took place mainly over land, and that gene flow among beaches was predominantly historic. Extensive breaks in habitat continuity, such as sea cliffs, appear to be effective dispersal barriers to maintain contemporary separation between diverged populations. Conversely, continuous beaches facilitate dispersal and connectivity among habitat patches. Pill bugs occur as naturally fragmented populations, thus increasing their risk of extirpation, but anthropogenically driven habitat fragmentation superimposed on sea-level rise

would further increase the risk of extinction in this species. Representative protection of the entire littoral active zone is imperative for the survival of this, and other, supralittoral species.

Acknowledgements. This study was funded by the National Research Foundation (NRF), the Applied Centre for Climate and Earth System Sciences (ACCESS). Staff of the South African National Parks, Woody Cape Section, are also gratefully acknowledged for logistical support.

LITERATURE CITED

Baker MB, Rao S (2004) Incremental costs and benefits shape natal dispersal: theory and example with *Hemilepistus reaumuri*. Ecology 85:1039–1051

Baldanzi S, Gouws G, Barker NP, Fratini S (2016) Molecular evidence of distinct evolutionary units in the sandhopper *Talorchestia capensis* (Amphipoda, Talitridae) along South African coasts. Hydrobiologia 779:35–46

Beerli P (2009) How to use MIGRATE or Why are Markov chain Monte Carlo programs difficult to use? In: Bertorelle G, Bruford MW, Hauffe HC, Rizzoli A, Vernesi C (eds) Population genetics for animal conservation. Cambridge University Press, Cambridge, p 42–79

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann Stat 29:1165–1188

Bird E (2000) Coastal geomorphology: an introduction. John Wiley, Chichester

Brown A (2000) Is the sandy-beach isopod *Tylos granulatus* an endangered species? S Afr J Sci 96:466

Brown AC, McLachlan A (2006) The ecology of sandy shores, 2nd edn. Academic Press, London

Brown A, Odendaal F (1994) The biology of oniscid Isopoda of the genus *Tylos*. Adv Mar Biol 30:89–153

Compton JS (2011) Pleistocene sea-level fluctuations and human evolution on the southern coastal plain of South Africa. Quat Sci Rev 30:506–527

Coupland GT, Duarte CM, Walker DI (2007) High metabolic rates in beach cast communities. Ecosystems 10: 1341–1350

De Matthaeis E, Ketmaier V, Davolos D, Schembri P (2000) Patterns of genetic diversity in Mediterranean supralittoral amphipods (Crustacea, Amphipoda). Pol Arch Hydrobiol 47:473–487

Defeo O, Gómez J (2005) Morphodynamics and habitat safety in sandy beaches: life-history adaptations in a supralittoral amphipod. Mar Ecol Prog Ser 293:143–153

Defeo O, McLachlan A (2011) Coupling between macrofauna community structure and beach type: a deconstructive meta-analysis. Mar Ecol Prog Ser 433:29–41

Defeo O, McLachlan A, Schoeman DS, Schlacher TA and others (2009) Threats to sandy beach ecosystems: a review. Estuar Coast Shelf Sci 81:1–12

Deidun A, Bonavia FG, Schembri PJ (2011) Distribution of *Tylos* spp. in the Maltese Islands and population dynamics of *Tylos europaeus*. J Coast Res 64:369–372

Dingle R, Rogers J (1972) Effects of sea-level changes on the Pleistocene palaeoecology of the Agulhas Bank. Palaeoecol Afr 6:55–58

Dugan JE, Hubbard DM, Rodil IF, Revell DL, Schroeter S (2008) Ecological effects of coastal armoring on sandy beaches. Mar Ecol 29:160–170

Dugan JE, Defeo O, Jaramillo E, Jones AR and others (2010) Give beach ecosystems their day in the sun. Science 329: 1146

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564–567

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Felsenstein J (1989) PHYLIP 3.2 manual. University of California Herbarium, Berkeley, CA

Folmer O, Black M, Hoech W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Gaston KJ, Duffy JP, Bennie J (2015) Quantifying the erosion of natural darkness in the global protected area system. Conserv Biol 29:1132–1141

Giribet G, Carranza S, Baguna J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Mol Biol Evol 13:76–84

Gonçalves SC, Pardal MA, Cardoso PG, Ferreira SM, Marques JC (2005) Biology, population dynamics and secondary production of *Tylos europaeus* (Isopoda, Tylidae) on the western coast of Portugal. Mar Biol 147: 631–641

Greenan TM, Griffiths CL, Santamaria CA (2018) Molecular approaches uncover cryptic diversity in intertidal *Ligia isopods* (Crustacea, Isopoda, Ligiidae) across the southern Africa coastline. PeerJ 6:e4658

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol 66:591–600

Harris L, Nel R, Schoeman D (2011) Mapping beach morphodynamics remotely: a novel application tested on South African sandy shores. Estuar Coast Shelf Sci 92:78–89

Harris L, Campbell EE, Nel R, Schoeman D, Beaumont L (2014) Rich diversity, strong endemism, but poor protection: addressing the neglect of sandy beach ecosystems in coastal conservation planning. Divers Distrib 20: 1120–1135

Hart MW, Marko PB (2010) It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. Integr Comp Biol 50:643–661

Hubbard DM, Dugan JE, Schooler NK, Viola SM (2014) Local extirpations and regional declines of endemic upper beach invertebrates in southern California. Estuar Coast Shelf Sci 150:67–75

Hübner L, Pennings SC, Zimmer M (2015) Sex-and habitat-specific movement of an omnivorous semi-terrestrial crab controls habitat connectivity and subsidies: a multiparameter approach. Oecologia 178:999–1015

Hurst GDD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc R Soc B 272:1525–1534

Hurtado LA, Lee EJ, Mateos M (2013) Contrasting phylogeography of sandy vs. rocky supralittoral isopods in the megadiverse and geologically dynamic Gulf of California and adjacent areas. PLOS ONE 8:e67827

Hurtado LA, Lee EJ, Mateos M, Taiti S (2014) Global diversification at the harsh sea–land interface: mitochondrial phylogeny of the supralittoral isopod genus *Tylos* (Tylidae, Oniscidea). PLOS ONE 9:e94081

Illenberger WK, Rust IC (1988) A sand budget for the Alexandria coastal dunefield, South Africa. Sedimentology 35:513–521

Kensley BF (1974) Aspects of the biology and ecology of the genus *Tylos* Latreille. Ann S Afr Mus 65:401–471

Ketmaier V, Scapini F, De Matthaeis E (2003) Exploratory analysis of talitrid population genetics as an indicator of the quality of sandy beaches. Estuar Coast Shelf Sci 58: 159–167

Ketmaier V, Iuri V, De Matthaeis E (2005) Genetic resources and molecular markers in *Talitrus saltator* (Amphipsoda, Talitridae) from the beach of Smir. In: Bayed A, Scapini F (eds) Ecosystèmes côtiers sensibles de la Méditerranée: cas du littoral de Smir. Travaux de l'Institut Scientifique, Rabat, série générale 4:55–59

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Can J Zool 85:1049–1064

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? Mol Ecol 19:3038–3051

Lowman HE, Emery KA, Kubler-Dudgeon L, Dugan JE, Melack JM (2019) Contribution of macroalgal wrack consumers to dissolved inorganic nitrogen concentrations in intertidal pore waters of sandy beaches. Estuar Coast Shelf Sci 219:363–371

Luarte T, Bonta C, Silva-Rodriguez E, Quijón P, Miranda C, Farias A, Duarte C (2016) Light pollution reduces activity, food consumption and growth rates in a sandy beach invertebrate. Environ Pollut 218:1147–1153

Markow TA, Pfeiler E (2010) Mitochondrial DNA evidence for deep genetic divergences in allopatric populations of the rocky intertidal isopod *Ligia occidentalis* from the eastern Pacific. Mol Phylogenet Evol 56:468–473

Mattos G, Seixas VC, Paiva PC (2019) Comparative phylogeography and genetic connectivity of two crustacean species with contrasting life histories on South Atlantic sandy beaches. Hydrobiologia 826:319–330

Mbongwa NA, Hui C, Pulfrich A, von der Heyden S (2019) Every beach an island — deep population divergence and possible loss of genetic diversity in *Tylos granulatus*, a sandy shore isopod. Mar Ecol Prog Ser 614:111–123

McLachlan A (1991) Ecology of coastal dune fauna. J Arid Environ 21:229–243

McLachlan A, Defeo O, Short AD (2018) Characterising sandy beaches into major types and states: implications for ecologists and managers. Estuar Coast Shelf Sci 215: 152–160

Menzies RJ (1952) The occurrence of a terrestrial isopod in plankton. Ecology 33:303

Merritt TJS, Shi L, Chase MC, Rex MA, Etter RJ, Quattro JM (1998) Universal cytochrome b primers facilitate intraspecific studies in molluscan taxa. Mol Mar Biol Biotechnol 7:7–11

Narum SR (2006) Beyond Bonferroni: less conservative analyses for conservation genetics. Conserv Genet 7:783–787

Niikura M, Honda M, Yahata K (2015) Phylogeography of semiterrestrial isopod, *Tylos granuliferus*, on East Asian coasts. Zool Sci 32:105–113

Odendaal F, Eekhout S, Brown A, Branch G (1999) Aggregations of the sandy-beach isopod, *Tylos granulatus*: adaptation or incidental-effect? Afr Zool 34:180–189

Partridge T, Scott L, Hamilton J (1999) Synthetic reconstructions of southern African environments during the Last Glacial Maximum (21–18 kyr) and the Holocene Altithermal (8–6 kyr). Quat Int 57-58:207–214

Pavesi L, Ketmaier V (2013) Patterns of genetic structuring and levels of differentiation in supralittoral talitrid amphipods: an overview. Crustaceana 86:890–907

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269–297

Pilkey OH, Cooper JAG (2014) Are natural beaches facing extinction? J Coast Res 70:431–436

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. Mol Biol Evol 19:2092–2100

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Roberts CM, Hawkins JP (1999) Extinction risk in the sea. Trends Ecol Evol 14:241–246

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Salzburger W, Ewing GB, Von Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. Mol Ecol 20:1952–1963

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562

Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc Natl Acad Sci USA 105:13486–13491

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Takada Y, Sakuma K, Fujii T, Kojima S (2018) Phylogeography of the sandy beach amphipod *Haustorioides japonicus* along the sea of Japan: paleogeographical signatures of cryptic regional divergences. Estuar Coast Shelf Sci 200:19–30

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases. Mol Biol Evol 9:678–687

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729

Toms JA, Compton JS, Smale M, von der Heyden S (2014) Variation in palaeo-shorelines explains contemporary population genetic patterns of rocky shore species. Biol Lett 10:20140330

Tourinho JL, Márquez A, Celentano E, Defeo O (2016) A new evolutionary unit in the *Excirolana braziliensis* species complex. Braz J Oceanogr 64:197–202

Van Andel TH (1989) Late Pleistocene sea levels and the human exploitation of the shore and shelf of southern South Africa. J Field Archaeol 16:133–155

Varela AI, Haye PA (2012) The marine brooder *Excirolana braziliensis* (Crustacea: Isopoda) is also a complex of cryptic species on the coast of Chile. Rev Chil Hist Nat 85:495–502

von der Heyden S (2009) Why do we need to integrate population genetics into South African marine protected area planning? Afr J Mar Sci 31:263–269

von der Heyden S, Beger M, Toonen RJ, van Herwerden L and others (2014) The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bull Mar Sci 90:123–158

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82: 117–125

Wright D, Bishop JM, Matthee CA, von der Heyden S, Burridge C (2015) Genetic isolation by distance reveals restricted dispersal across a range of life histories: implications for biodiversity conservation planning across highly variable marine environments. Divers Distrib 21:698–710

Editorial responsibility: Inna Sokolova, Rostock, Germany
Reviewed by: 2 anonymous referees

Submitted: February 13, 2021
Accepted: July 22, 2021
Proofs received from author(s): September 13, 2021