When assessing genetic structuring within each landmass, each sampling locality was treated as a separate unit. Locations with <2 individuals were excluded from the analyses. To examine the correlation between the 2 types of marker at the level of individual slugs, individual pairwise distance matrices were constructed using only those individuals for which both COI and AFLP data were available (n = 55, Table S2 in the Supplement). To compare levels of genetic diversity, summary statistics were calculated using Arlequin 3.5.1.3 (Excoffier & Lischer 2010) and GenAlEx 6.14 (Peakall & Smouse 2006).

RESULTS

mtDNA phylogeography of *Onchidella nigricans*

The most immediate feature of the mtDNA haplotype network of *Onchidella nigricans* (COI) is the close relationships observed between all 80 haplotypes (n = 111) (Fig. 2). Average divergence between COI haplotypes of individual slugs was 0.8%, with most haplotypes separated by 1 or 2 base pair differences. No obvious geographic clustering according to landmass was detected (Fig. 2, Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m562p093_supp.pdf). Of the 80 haplotypes, 8 were detected in multiple localities; 5 of them were shared across the Tasman Sea, and 7 between New Zealand's North and South Islands. The most commonly shared haplotype (NH1) was found in 16% of all individuals (11 different localities spanning all 3 landmasses) (Fig. S1a, Table S2). For 16S rDNA, a similarly geographically unstructured tree topology was obtained (Fig. S1b) with equally low average divergence among haplotypes (average 0.7%). Haplotype relationships within *O. nigricans* were generally poorly resolved, with low bootstrap support for most branches (Fig. S1).

Local diversity levels in mtDNA and AFLP fingerprint data

Haplotype diversity (mtDNA) values within localities ranged from 0.83 to 1 and nucleotide diversity ranged from 2.86 to 10.4, reflecting a high number of unique, but consistently similar, haplotypes (Table S3, Fig. 2). In AFLP data, the mean expected heterozygosity (H_E) by location ranged from 0.057 to 0.127 (Table S4). For landmass, the North Island recorded the lowest average H_E values (0.075), followed by Australia (0.090) and the South Island (0.099) (Table S4). Eden recorded the highest number of loci (152) and private bands (31). However, low sample sizes for AFLP data call for caution when drawing conclusions regarding H_E.

Spatial partitioning of genetic variation and differentiation between landmasses

For mitochondrial COI, most of the variation was detected within sampling localities (98%), compared to 2% among landmasses and 0% of variation between localities (Table S5a). Overall, landmasses were significantly differentiated (Φ_{PT} = 0.023; p = 0.018). Φ_{PT} measures the relative contribution of between-unit variation to the overall variation in the whole sample, taking into account both haplotype and nucleotide diversity in the data. In pairwise comparisons, weak but significant landmass-level structuring was detected across the Tasman Sea, with the Australian samples significantly differentiated from both New Zealand's North (Φ_{PT} = 0.058; p = 0.009) and South Islands (Φ_{PT} = 0.058; p = 0.011) (Table S6a). In contrast, New Zealand's North and South Island sample pools were not significantly differentiated from each other (Φ_{PT} = 0.000; p = 0.374). As expected with 0% variation among populations in COI data, the principal component analysis (PCA) plot of locality pairwise Φ_{PT} values places most locations in a compact cluster along the main axis (Fig. S2a). Only the Australian population of Eden is positioned far away from the rest of the localities on the primary axis. Correspondingly, Eden was significantly differentiated from 6 localities, all of them from New Zealand; no other significantly different locality pairs were found in the COI data (Table S7).

AFLP analyses revealed more substantial landmass- and locality-level differentiation than mtDNA. Although 97% of variation was found within localities, a small but significant portion of variation was distributed among landmasses (3%) (localities 0%) (Table S5b). Overall, landmasses were significantly differentiated (Φ_{RT} = 0.028; p = 0.0002). In pairwise comparisons of AFLP data, the Australian samples were significantly differentiated from the South Island (Φ_{PT} = 0.025; p = 0.003) but not from the North Island samples (Φ_{PT} = 0.025; p = 0.458) (Table S6b, Fig. S2b). In contrast to the COI data, New Zealand's North Island samples were significantly different from the South Island samples (Φ_{PT} = 0.062; p = 0.0001) (Table S6b, Fig. S2). Reflective of this finding, AFLP fingerprint distances between individual slugs in a PCA plot similarly stretch Australia and South Island individuals

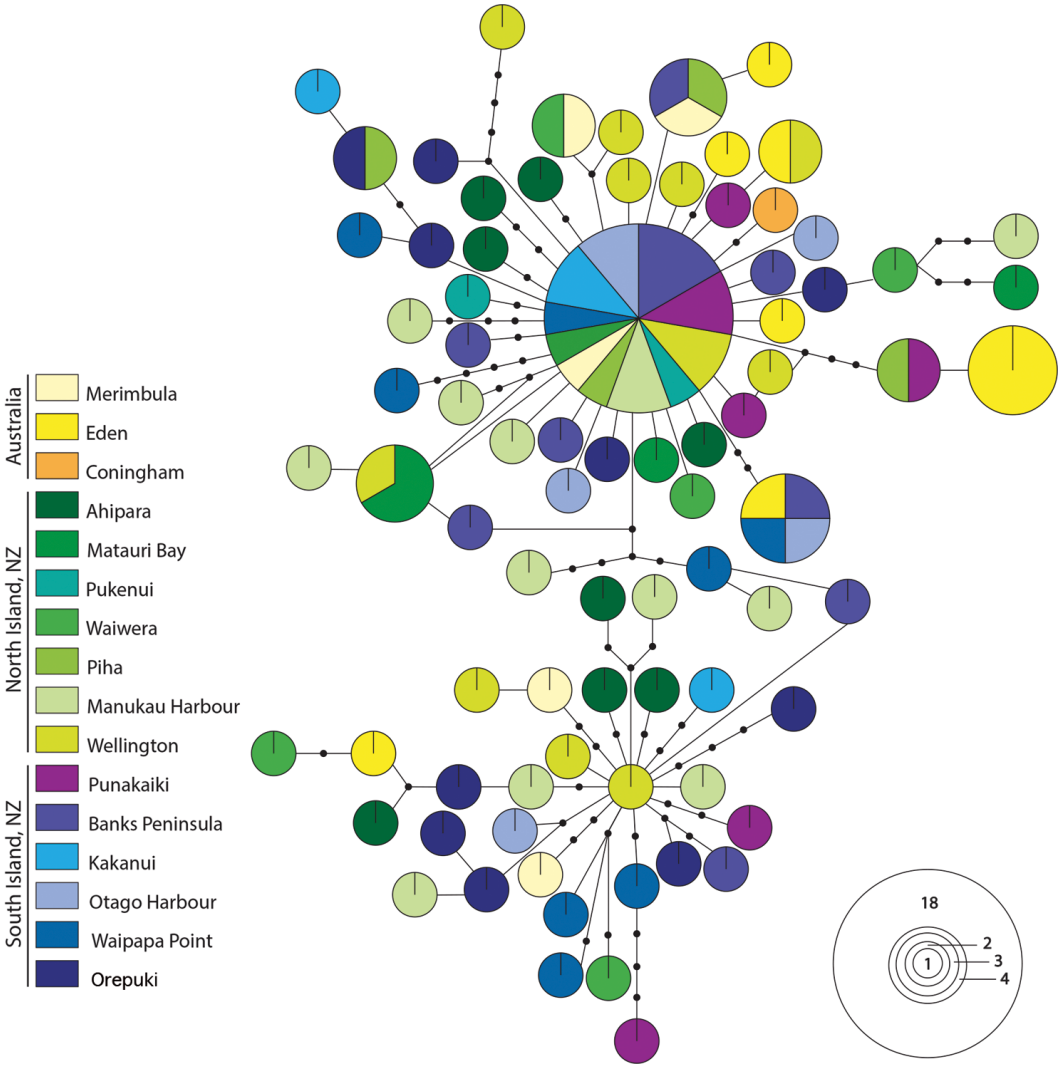

Fig. 2. Statistical parsimony network of mitochondrial COI haplotypes detected for *Onchidella nigricans*. The area of each circle/slice reflects the number of individuals detected with the haplotype (see scale), and the colours indicate their geographical origin. Hypothetical intermediate haplotypes not detected in the current study are indicated (●)

the full length of the *x*-axis in contrast to the North Island, which is clustered to the right (Fig. S3). North Island and South Island samples are almost completely separated along the *x*-axis in a PCA plot (Fig. S2b), and the east coast South Island samples of Banks Peninsula, Kakanui and Otago Harbour were often significantly differentiated in pairwise comparisons with other localities (Table S7). However, the low per-locality sample sizes (typical n = 2 to 5) in AFLP data preclude any solid conclusions about between-locality structuring (Table S8). The correlation of the locality pairwise Φ_{PT} estimates calculated from AFLP data with corresponding estimates from mtDNA data was not significant ($R^2 = 0.003$; $p = 0.308$).

DISCUSSION

Both AFLP and mtDNA markers indicate an unexpectedly low degree of genetic structuring or differentiation across the sampling region, especially considering the expansive geographic range of *Onchidella nigricans*. This 'starburst' type genetic pattern (Fig. 2) — often reflective of recent coalescence, population expansion or ongoing dispersal (Templeton 1998) — contrasts significantly with that of the encapsulated-developing congener *O. marginata*, whose population genetic pattern was attributed to its ecology (associated with buoyant macroalgae) and reproductive life history (Cumming et al. 2014).

Ongoing gene flow from planktonic larval dispersal?

One might argue that the low genetic structuring across the sampling range of *O. nigricans* is due to dispersal by planktotrophic larvae. However, while a larval duration of 3 wk would provide for genetic connectivity within New Zealand (Chiswell & Rickard 2011), it seems insufficient for crossing the Tasman Sea. If larvae adhered strictly to a larval duration of 3 wk, based on the commencement of settling behaviour (Stringer 1962), then strong genetic differentiation would be expected across the Tasman Sea and elsewhere across its broad distribution (Bohonak 1999). Our results are in contrast to this expectation, as connectivity rather than isolation is reflected in the data. Thus, if *O. nigricans* larvae are regularly crossing the Tasman Sea, larval development times are much longer and/or ocean crossing times are much shorter than expected.

Theoretically, an object travelling at the same speed as the ocean current could cross the Tasman Sea in approximately 100 to 200 d (direct line minimum crossing based on an estimated mean current speed of 10 to 20 cm s^{-1} [Chiswell 2016] and 1800 km gap). However, transit times are very unlikely to be this short under natural conditions, with oceanographic simulations suggesting that the median time for passively drifting larvae to reach New Zealand from southern Australia is 1099 d (Chiswell et al. 2003). Even the fastest percentile crossing time of 393 d (Chiswell et al. 2003) seems too long to explain the genetic pattern we observed. However, these times do not account completely for rare and extreme weather events, which could provide highly favourable conditions resulting in occasional 'connectivity bursts'. During such bursts, taxa that are thought to be unable to cross the Tasman Sea (based on PLD and estimated crossing times) might, in fact, do so. Oceanic variables (e.g. current speed, eddies) and geographic variation (e.g. distance between landmasses) would also have implications for larvae released in different parts of the range of *O. nigricans* (Fig. 1). The significant population-level divergence observed in our data often involved Eden (Table S7), which is located in the southern coast of mainland Australia — an area of high eddy variability. If the coastline of this area facilitates larval retention and local recruitment, as suggested by Banks et al. (2007) in a study of the broadcast-spawning sea urchin *Centrostephanus rodgersii*, this could be reflected in our data. Consequently, some populations may be better placed geographically to serve as a potential source from Australia to New Zealand, particularly those closer to the Tasman Front (Chiswell & Booth 2008). Further studies with more intensive sampling would be useful to confirm such localized effects on dispersal.

A process of stepping-stone dispersal, whereby *O. nigricans* maintained populations on the geographically intermediate oceanic islands of Lord Howe Island and Norfolk Island, would help explain the suggested pattern of connectivity. However, *O. nigricans* is notably absent from both islands, despite potentially suitable habitat being available. The lack of a suitably long and sheltered bay to retain larvae locally could prevent the establishment of self-recruiting, persistent populations. Moreover, if local retention is not possible, then these islands may be too far from Australian mainland populations and too small a 'target' to enable regular recruitment. Consequently, a 'paradox of Rockall' scenario might explain their absence (Johannesson 1988, Cumming 2013); in support of this possibility, *O. nigricans* is also absent from the Chatham Islands (despite being erroneously recorded; R. Cumming pers. obs.) and Stewart Island in New Zealand.

Nevertheless, even if exceptionally fast crossings do occasionally occur, they are more likely to be in the range of months than weeks, so further explanation is needed. It could be that *O. nigricans* larvae are physiologically able to extend their PLD well beyond the time at which Stringer (1962) first observed settlement behaviour in actively feeding larvae. Many taxa can extend PLD by delaying settlement and metamorphosis in the absence of a suitable substratum or settlement cue (Hadfield 1998). Indeed, the frequent occurrence of delayed settlement ability could be expected if it provides an adaptive advantage over strictly constrained PLD, thereby increasing the opportunity for successful settlement. Therefore, to predict the magnitude of gene flow, understanding minimum and maximum PLD is more informative (Weersing & Toonen 2009). With a PLD upper limit of months rather than weeks, the likelihood of *O. nigricans* being able to cross the Tasman Sea becomes more feasible and fits with patterns observed in other taxa. For example, the rocky shore snail *Nerita melanotragus* exhibits no significant differences between eastern Australia and New Zealand at the mtDNA COI gene (Waters et al. 2005; see Spencer et al. 2007 for the correct taxonomy) and does not have an exceptionally long larval duration (PLD 5 to 6 mo).

Larval duration, although critical, is unlikely to be the only factor that determines population structure for marine invertebrates with planktonic larvae. Even taxa with seemingly similar PLD can show con-

trasting patterns of differentiation and, in such cases, other life history factors should be considered (e.g. larval behaviour, fecundity) (Shanks 2009). For example, the rock lobster *Sagmariasus verreauxi* has slightly longer PLD (>8 mo; Brasher et al. 1992) than *N. melanotragus*, but does show genetic differentiation across the Tasman Sea, possibly due to differences in larval behaviour (Chiswell et al. 2003). Also, *N. melanotragus* adults can be very abundant (50 to 140 m^{-2}), so a high number of adults collectively releasing enormous numbers of offspring (Underwood 1974) could result in a small but non-negligible degree of trans-Tasman recruitment. Similarly, *O. nigricans* is highly fecund (500 to 11 000 eggs per mass; Stringer 1962) and often is locally abundant (R. A. Cumming pers. obs.). Hence, these life history traits together with occasional 'connectivity bursts' could be sufficient to satisfy the 'one migrant per generation rule' needed to maintain genetic continuity (Mills & Allendorf 1996).

Clearly, estimates of larval duration alone are not sufficient to predict genetic continuity across the Tasman Sea, especially as there is also considerable intraspecific variation in PLD, even within the same brood (Hadfield & Strathmann 1996). Results from our study imply that caution is needed when applying estimates of PLD obtained from laboratory experiments to natural populations. Indeed larval rearing experiments are notoriously difficult and provide only a limited range of possible conditions; thus, the potential to underestimate PLD is high. For example, Booth & Phillips (1994) found that the duration of the phyllosoma larval stage for the rock lobster *Jasus edwardsii* under natural conditions (~12 to 24 mo) was underestimated in aquaria by about half (~10 to 11 mo).

Alternative dispersal mechanisms: rafting or human-mediated translocation?

While an extended PLD seems the most likely explanation for the high genetic connectivity observed in *O. nigricans*, dispersal by other means (e.g. rafting or human translocation) requires consideration. For example, kelp-associated taxa can extend dispersal distance beyond the constraints of PLD by rafting on detached macroalgae (Thiel & Gutow 2005). Although most commonly found on rocks, *O. nigricans* is often found in areas where the intertidal fucoid algae *Hormosira banksii* is abundant (R. A. Cumming pers. obs.) (Fig. 1d). The thalli of *H. banksii* consist of branched chains of fluid-filled vesicles that are able to float and disperse small distances if detached (McKenzie & Bellgrove 2008). Although rafting on *H. banksii* (or some other buoyant substrate) could potentially provide an occasional dispersal opportunity, it seems unlikely to be a regular dispersal mechanism for 2 reasons. First, it would be challenging for a slug to remain attached to drifting *H. banksii* for extended periods of time, as its thallus structure offers no protection from water movement or predators (e.g. pelagic fishes). Second, *H. banksii* itself has a strong pattern of isolation-by-distance along the east coast of Australia (Coleman et al. 2011), indicating that its ability to generate high gene flow in its own or other hitchhiking species is limited.

Based on the low level of genetic structuring across the Tasman Sea, the possibility that anthropogenic translocation has enabled *O. nigricans* to expand its range fairly recently (i.e. the past 100 yr) is plausible, particularly as there are numerous examples of human-mediated introductions of marine invertebrates in temperate Australasia (Hewitt et al. 2004). However, like rafting, human introduction is unlikely to explain the broad connectivity observed for *O. nigricans*, as local levels of mtDNA and AFLP diversity are consistently high in both Australian and New Zealand populations of the species (Tables S3 & S4). Had recent anthropogenic introduction of *O. nigricans* occurred from New Zealand to Australia or vice versa, reduced diversity in areas recently colonised relative to the native range would generally be expected (Roman & Darling 2007). It is also plausible that *O. nigricans* underwent recent independent range expansion or had an unstable population history in Australasia (e.g. widespread Pleistocene extinction followed by wholesale recolonisation), as both of these scenarios could result in the shallow differentiation detected throughout the sampled areas (Marko et al. 2010, Cumming et al. 2014). However, once again, high local diversity contradicts this scenario and an effective means of dispersal would still be required to facilitate widespread recolonisation/expansion during such events.

In conclusion, the planktonic development of *O. nigricans* appears to facilitate surprisingly high connectivity throughout its broad geographic range, and particularly across the 1500 to 2000 km stretch of ocean separating Australia and New Zealand. An extended larval duration and/or delayed larval settlement seem the most likely hypotheses to explain this unexpected pattern. Importantly, population genetic analyses such as this can provide a strong rationale for subsequent reassessment of invertebrate life histories, particularly if they are used as a basis for inferring connectivity.

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