

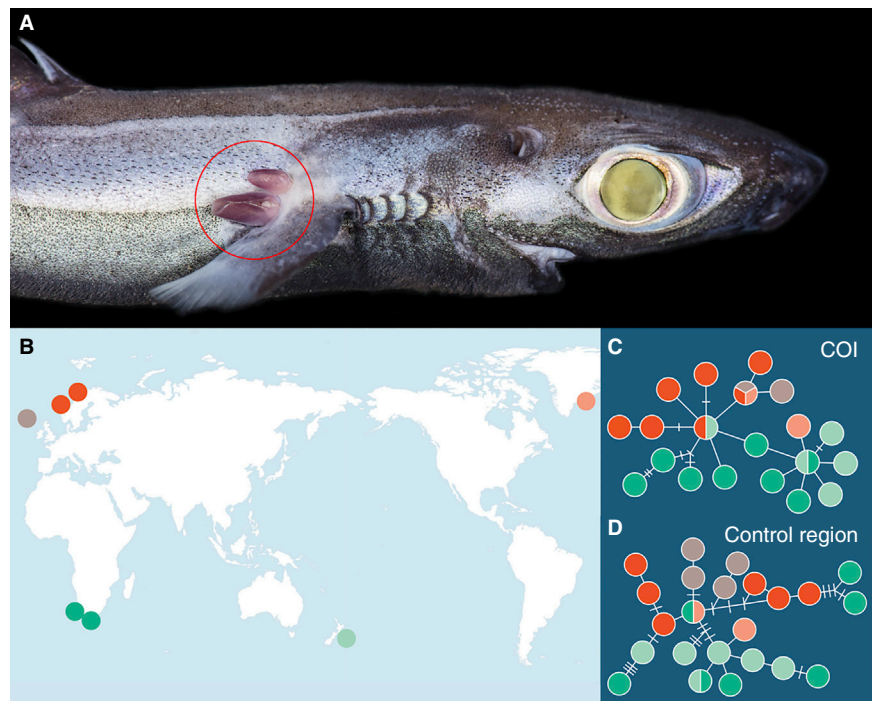
## Correspondence

**De novo innovation allows shark parasitism and global expansion of the barnacle *Anelasma squalicola***

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The barnacle *Anelasma squalicola* is a marine epibiont found on members of the species-rich, deep-sea lantern shark family Etmopteridae (Figure 1A) but is unlike any other epibiotic thoracian barnacles [1]. While many barnacle species are associated with various marine animals including turtles and whales, with the exception of *Anelasma* these all retain a filter-feeding lifestyle and have a commensal relationship with their host; despite often being deeply embedded in the dermis, no other species has been reported as feeding on its host. Although *Anelasma* is fully equipped with cirri (thoracic appendages), these are no longer used for filter feeding [1]. Instead, *Anelasma* embeds a stalk with root-like structures into the flesh of the shark (Figure S1C in Supplemental Information, published with this article online) that it uses to parasitize its host. Here, we show that specimens of *Anelasma* sampled from all over the world show very little genetic differentiation, suggesting that this innovation coincided with a rapid worldwide expansion.

Stable isotope comparisons between *Anelasma*, its shark host, and a conventional suspension feeding barnacle indicate that *Anelasma* takes nutrition directly from its host shark, via the stalk, and, unlike its barnacle relatives, not from zooplankton in the ambient sea water. This is remarkable since an apparently functional alimentary tract, including accessory glands, is present, although the stomach, in contrast to filter feeding barnacles, is consistently found empty



**Figure 1. Anatomy and haplotype network of the shark barnacle *Anelasma squalicola*.**

(A) Specimen of the velvet belly lantern shark, *Etmopterus spinax*, with a pair of *A. squalicola* embedded at the base of the pectoral fin (encircled). (B) Global map with *A. squalicola* sampling locations. (C,D) Haplotype networks for COI (C) and the control region (D) colour coded by sampling site in (B).

[2]. The mouthparts and cirri are also still present, but asymmetrical [2] and reduced (Figure S1A). When large, energy-consuming key organ systems, such as these, are rendered obsolete and do not perform other functions for the organism, natural selection is expected to lead to rapid reduction. The fact that these structures still exist in *Anelasma* indicates that the stalk feeding system only recently took over the nutrient absorption function from the filter feeding apparatus. A filter-feeding lifestyle has been hypothesised to represent an unlikely potential starting point for a transition from free-living to parasitism [3], which perhaps makes *Anelasma* especially interesting.

Through years of intensive collecting activities, we have sampled parasitized shark species across the barnacle's global distribution range (Figure 1B). With such a wide distribution, regional speciation or clear differentiation between populations would normally be expected [4]. Analyzing data from five molecular markers (nuclear ribosomal 18S and 28S, and mitochondrial 16S, Cytochrome c oxidase subunit 1 (COI)

and the control region) showed that despite the enormous geographic distances, and the fact that at least 11 different shark species act as hosts (Table S1), extremely little genetic variation exists. No sequence differences were found in 18S or 28S, while 16S exhibited minimal variation; two specimens from South Africa differed from all others by a single substitution. The two remaining mitochondrial genes demonstrated more variation; 20 haplotypes were found among 67 individuals for COI, and 23 haplotypes for the control region from 41 specimens; no marker indicated any geographic pattern, illustrated in the haplotype networks in Figure 1C,D. To put this lack of variation in context, population genetic studies of two widely distributed intertidal barnacle species, *Amphibalanus improvisus* (temperate) and *Balanus amphitrite* (subtropical), have revealed high levels of genetic diversity for COI and significant genetic differentiation in populations throughout their home ranges, as well as in introduced regions [5]. Despite larvae with significant

dispersal ability and large continuous populations in their home ranges, no panmixia exists and genetically differentiated populations are apparently continuously developing due to ongoing isolation by distance processes. In *Anelasma* we find low genetic diversity and no geographic differentiation despite the fact that the distribution range of this species is larger than in both *A. improvisus* and *B. amphitrite*. In molecular analyses (including COI) of the epipelagic, pedunculated rafter barnacle genus, *Lepas*, Schiffer and Herbig [6] found that the previously acknowledged cosmopolitan species, *Lepas anatifera*, could be divided into four regional subgroups, in addition to a global group. They also identified two species, *L. pectinata* and *L. anserifera*, which appeared to have a true worldwide oceanic distribution. However, *L. pectinata* and *L. anserifera* are extreme ecological generalists known for being capable of long-distance dispersal (using the great surface currents of the world oceans) and, therefore, possibly able to maintain gene flow, even with a cosmopolitan distribution.

The global distribution of *Anelasma* is highly unlikely to represent a single interbreeding population. Although we cannot completely discount the possibility of gene flow over large distances via a stepping-stone system involving overlapping host distributions, several factors suggest that this is unlikely. *Anelasma nauplii* larvae are poor swimmers and cannot efficiently move through the water column (Video S1), directly limiting larval dispersal. While there is overlap in distributions of some known hosts over large geographic areas (e.g. South Africa to New Zealand), the lack of dispersal ability of *Anelasma* means that ‘leapfrogging’ involving multiple hosts worldwide would be required for a panmictic *Anelasma* population. Little is known about dispersal of deep-sea lanternsharks and some of the shark hosts involved may have limited dispersal behaviour, which would further constrain *Anelasma* movement over large distances. This is supported by the fact that we observe high prevalence (ca. 20%) of *Anelasma* on *Etmopterus spinax* in one side-fjord of the Sognefjord but have never found any *Anelasma*

on *Etmopterus* in a parallel fjord just 20 km away (or in numerous other local fjords). The apparent difficulty in *Anelasma* dispersal over such modest scales makes a stepping-stone dynamic, involving multiple shark populations from Norway to New Zealand (a distance of 23,000 km), an unlikely scenario for a global panmictic *Anelasma* population. While we cannot rule out some potential for genetic exchange over large distances, absence of larval dispersal capacity, low population densities, patchy distribution and reliance on a stepping-stone system of dispersal via various shark hosts would seem to make for an inefficient system for maintenance of a global *Anelasma* population, hence our view that the observed genetic pattern is better explained by a recent (in evolutionary terms) expansion event.

Based on the theory that the origin of such a population expansion is where haplotype diversity is greatest, our study suggests the southern Atlantic as a likely original population source. Multiple host species’ distributions overlap in waters around South Africa, including two with enormous ranges in the southern hemisphere (Table S1), and this region potentially bridges to the northern hemisphere through a common eastern Atlantic host species (*E. spinax*). In attempting to estimate time of the earliest haplotype divergence (approximate time of origin of *Anelasma* as a new species), we obtained from the literature maximum and minimum values for COI and control region mutation rates for related barnacle species, where fossil records or geographical events allowed reliable estimates [7]. The calculated divergence times were found to be between 274,000 and 545,000 ya for the COI gene, and between 408,000 and 811,000 ya for the control region (see Supplemental Experimental Procedures for details). This is remarkably recent in relation to the considerable time elapsed since the *Capitulum* (closest living relative to *Anelasma*) and the *Anelasma* lineages split in the Mesozoic, about 120 million years ago [1]. Furthermore, these COI and control region divergence times are conservative estimates due to the observation that parasites in general tend to display accelerated mutation rates [8]; the true divergence time is

probably considerably more recent. Thus, in brief: a rare macroevolutionary event, the *de novo* innovation of feeding via the stalk, prompted a global expansion of *Anelasma*. This happened so recently that there has not been enough time to develop distinct zoogeographical population identities, a pattern that, in fact, might apply to many presumed cosmopolitan species.

#### SUPPLEMENTAL INFORMATION

Supplemental Information contains one figure, one table, experimental procedures, and one video, all of which can be found with this article online at <https://doi.org/10.1016/j.cub.2019.04.053>.

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