

Molecular dating and biogeography of the neritic krill *Nyctiphanes*

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Abstract The genus *Nyctiphanes* (Malacostraca, Euphausiacea) comprises four neritic species that display antitropical geographic distribution in the Pacific (*N. simplex* and *N. australis*) and Atlantic (*N. couchii* and *N. capensis*) Oceans. We studied the origin of this distribution applying methods for phylogenetic reconstruction and molecular dating of nodes using a Bayesian MCMC analysis and the DNA sequence information contained in mtDNA 16S rDNA and cytochrome oxidase (COI). We tested hypotheses of vicariance by contrasting the time estimates of cladogenesis with the onset of the major barriers to ocean

circulation. It was estimated that *Nyctiphanes* originated in the Pacific Ocean during the Miocene, with a lower limit of 18 million years ago (Mya). An Atlantic–Pacific cladogenic event (95% HPD 3.2–9.6) took place after the closure of the Tethyan Sea, suggesting that dispersal occurred from the Indo-Pacific, most likely via southern Africa. Similarly, the antitropical distribution pattern observed in the eastern Atlantic Ocean likely resulted from recent Pliocene–Pleistocene (95% HPD 1.0–4.97) northward dispersal from the southern hemisphere. Our results imply that dispersal appears to have had a significant role to play in the evolution of this group.

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Introduction

Nyctiphanes is one of only two strictly neritic euphausiid genera in the Order Euphausiacea (Casanova 1984; van der Spoel et al. 1990; Maas and Waloszek 2001) and comprises four species that inhabit the productive temperate waters on either side of the equator in both the Pacific and Atlantic Oceans. They often dominate zooplankton biomass, thus forming the basis of economically important food chains (Pillar et al. 1992). Antitropical species distribution patterns such as that observed in *Nyctiphanes* can be explained by either founder dispersal or vicariance hypotheses of cladogenesis (Lindberg 1991 and references therein). We attempted to infer the most likely causes for the current disjunct geographic distribution patterns of *Nyctiphanes* spp. by dating the cladogenetic events of the reconstructed phylogeny of the group under different molecular clock models. If the fragmented distribution patterns in *Nyctiphanes* are the result of vicariance, the divergence time estimates should postdate the origin of the major barriers to dispersal; the closures of the Tethyan Sea ~14 million years ago

(Mya) (Rögl and Steininger 1983), the Indonesian Seaway \sim 13 Mya (White 1994), and the Central American Seaway \sim 3.1 Mya (Keigwin 1978). Founder dispersal was considered the most likely explanation for cladogenesis when species divergence time estimates were significantly different from the estimated dates of vicariant events documented in the literature. Vicariance versus dispersal hypotheses have been tested in other benthic (Teske et al. 2007 and references therein) and pelagic marine species (Bowen and Grant 1997; Grant and Bowen 1998). To our knowledge, this is the first study in which biogeographic hypotheses have been tested in euphausiids in a Bayesian framework under different evolutionary models.

Methods

The molecular phylogenetic relationships of *Nyctiphanes* spp. were reconstructed using several different methods of tree reconstruction and information from the mitochondrial cytochrome oxidase I gene (COI) and mitochondrial large subunit of the ribosomal RNA coding gene (16S rDNA). Molecular clock hypotheses were tested (Felsenstein 1981; Drummond et al. 2006) and molecular dating techniques using strict and relaxed molecular clock methods (Drummond et al. 2006) employed to estimate the divergence dates of species in the genus *Nyctiphanes*. We applied molecular rates from calibrated molecular clocks for crustacean COI and 16S (Schubart et al. 1998). A coestimate of nucleotide substitution model parameters, phylogeny and divergence was obtained using the Bayesian Markov chain Monte Carlo method implemented in BEAST v1.4.6 (Drummond and Rambaut 2006) employing probabilistic calibration prior to appropriately incorporate calibration uncertainties (Drummond and Rambaut 2006). This method is well suited to analyse the euphausiid

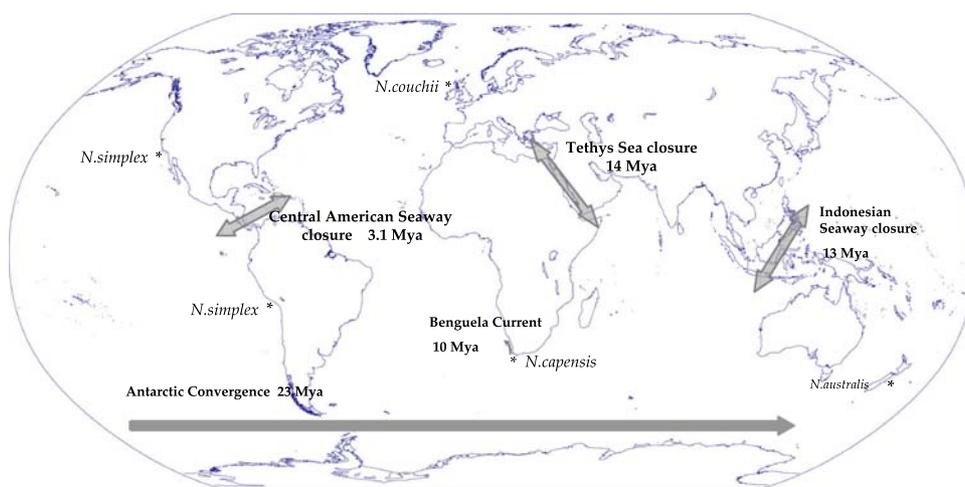
data given the absence of a fossil record and the substantially different body sizes observed among species in this group. The Yule process (Yule 1924) speciation model was used as a prior for the MCMC runs to infer the time to most common recent ancestor (TMCRA) in all the species-level analyses.

Nyctiphanes capensis Hansen was collected off South Africa and Namibia in February 2001, *N. couchii* Bell was collected off Scotland in 2001, *N. australis* G.O. Sars was collected off New Zealand in 2001, and *N. simplex* Hansen was collected off California in 2000 (Fig. 1).

Results

Congruent topologies were recovered in all the phylogenetic analyses irrespective of the gene fragment used, the method of tree construction employed, or the molecular clock model applied (Fig. 1, Supplementary Material). For the sake of brevity, only the Bayesian phylogeny of the 16 euphausiid species based on 16S is shown in Fig. 2. In all trees, the monophyly of *Nyctiphanes* was highly supported with both high bootstrap and clade credibility values recovered. This genus forms a subclade within a clade comprising the species *Meganyctiphanes norvegica*, *Nematoscelis megalops*, *Thysanoessa inermis*, *T. longipes* and *T. macrura*, and distinct from the clade containing the *Euphausia* species. This result is in agreement with a morphological assessment of the systematic position of this group (Casanova 1984; Maas and Waloszek 2001), who hypothesised that *Nyctiphanes* were closely related to *Meganyctiphanes* and *Pseueuphausia*, and *Nematoscelina* (*Thysanoessa*, *Stylocheiron*, *Nematoscelis* and *Tessarabrachion*) but not with the result of van der Spoel et al. (1990) who thought *Nyctiphanes* was more closely related to *Euphausia*.

Fig. 1 Geographic distribution of *Nyctiphanes* species. All sites except the southeast Pacific population of *N. simplex* were sampled. Semitransparent grey arrows indicate former open seaways; the onset of the current barriers is indicated in Mya (million years ago). Solid grey arrows indicate sea currents with their date of origin in Mya



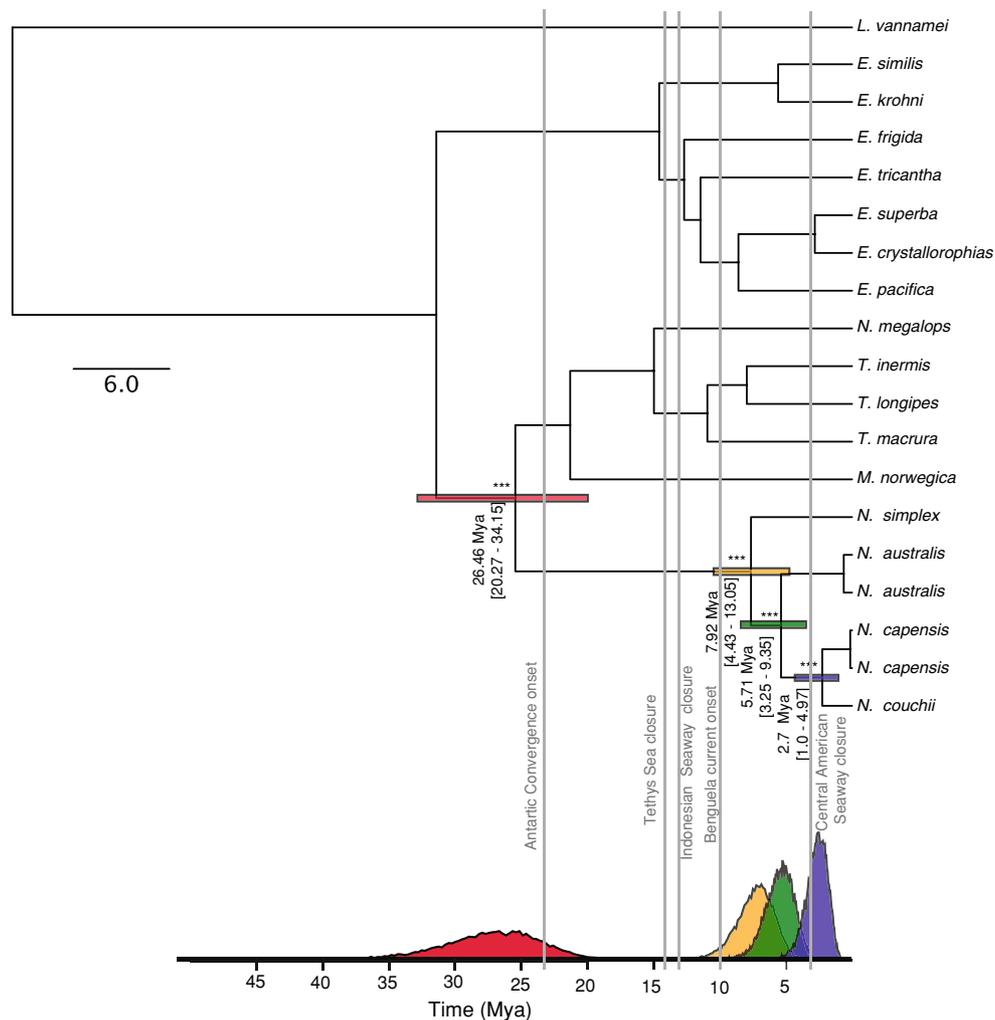


Fig. 2 The 16S consensus tree of the relaxed molecular clock analysis. The tips of the tree correspond to the sampled species in the present, branch lengths reflect the mean of the posterior probability density, i.e. internal nodes are scaled to time of origin. The posterior probability density for the time of most recent common ancestor (TMRCA) for the speciation of *N. couchii* and *N. capensis* is presented in dark blue, the TMRCA of the speciation event between *N. australis*, *N. capensis* and *N. couchii* is presented in green, the TMRCA of the speciation of *N. simplex* from the other three *Nyctiphanes* species presented is shown in

yellow and the TMRCA of the speciation event from the sampled *Nyctiphanes* and other euphausiids is shown in red. The TMRCA means and the 95% highest probability density (HPD) intervals is shown by the horizontal bars in the internal nodes of the *Nyctiphanes* clade and are indicated in text in the figures. Posterior probabilities > 0.9 are represented by triple asterisks in the internal nodes of the tree. This tree was calculated using the Yule speciation process and an evolutionary rate of 0.60% per million years were used as priors in the relaxed molecular clock analysis

Within the *Nyctiphanes* clade, *N. capensis* and *N. couchii* are inferred to be sister species to which *N. australis* is the sister taxon, and all three species share a common ancestor with *N. simplex* (Fig. 2). The intra- and interspecific nucleotide *p*-distances for both the data sets are available as supplementary material (Table 1, Supplementary Material).

Similar estimates of the TMRCA were recovered for both data sets under both the strict and relaxed molecular clock models with the widest 95% highest posterior density intervals (HPDs) obtained under the latter model (Table 2, Supplementary Material). Replicate MCMC runs in BEAST under the relaxed clock model estimate that the *Nyctiphanes* species shared a common ancestor around

7.924 Mya (95% HPD 4.431–13.059 Mya; 16S) and 9.815 Mya (95% HPD 5.748–18.149 Mya, COI) (Table 1). Under the same model, it was further estimated that *N. australis*, *N. capensis* and *N. couchii* shared an MRCA around 5.714 Mya (95% HPD 3.247–9.352 Mya, 16S) and 5.35 Mya (95% HPD 3.199–9.569 Mya, COI; Table 2 Supplementary Material). For the *N. australis* and *N. capensis* species pair, the TMRCA was estimated at 2.70 (95% HPD 1.002–4.973 Mya, 16S) and 2.575 Mya (95% HPD 1.377–4.977 Mya; COI). The values of the effective sample size (ESS) exceeded 9,000 for all runs, suggesting acceptable mixing and sufficient sampling of the MCMC chains (Rambaut and Drummond 2004).

Discussion

The similarity of the TMRCA estimates obtained for the different mitochondrial genes under both the strict and relaxed molecular clock models suggest that the signal for the TMRCA can be recovered even when the model may be misspecified. Almost identical results were obtained among replicate runs of the Bayesian MCMC analyses suggesting that tree space has been effectively sampled (Drummond et al. 2006). In all the phylogenetic analyses the Pacific species *N. simplex* and *N. australis* occupy the most basal branches in the *Nyctiphanes* clade, indicating that these are the oldest extant *Nyctiphanes* lineages.

If we assume that contemporary and ancestral geographical distributions are similar, this may imply an origin in the Pacific rather than in the Prototethys Sea during the Late and Post Cretaceous epochs (Van der Spoel et al. 1990). The onset of the Antarctic convergence ~23 Mya that led to the establishment of the temperate environment in the southern hemisphere (White 1994), precedes the date indicated for the *Nyctiphanes simplex* node in the phylogenetic trees (Fig. 2). Therefore, it is possible that *Nyctiphanes* originated in the south Pacific as a neritic krill group subsequent to the establishment of colder conditions along the highly productive coastlines that were thought to have existed in this region at this time (White 1994).

The establishment of a temperate environment in the North Pacific as a consequence of the closure of the Indonesian Seaway (White 1994), is thought to have occurred subsequent to that in the southern hemisphere. Thus the presence of *N. simplex* in the northern Pacific is possibly a result of recent dispersal from the south. Evidence for trans-tropical dispersal in the East Pacific at the time of the Pliocene–Pleistocene boundary has been well-documented for several taxa (Bowen and Grant 1997; Grant and Bowen 1998; Lindberg 1991; Dawson 1946; Valentine 1955; Emerson 1952).

The origin of the *N. australis* species lineage postdates the two major geotectonic events in the Miocene; the closures of the Tethyan and Indonesian seaways around 14 and 13 Mya, respectively (Rögl and Steininger 1983; White 1994), which led to the establishment of novel ocean circulation and climatic patterns. The opening of new environments may have influenced the origin and diversification of this group in the Indo-West Pacific and Indian Ocean, giving rise to the *N. australis* and proto-Atlantic lineages.

The sister species relationship between *N. australis* and the Atlantic species group suggests that the colonization of the Atlantic perhaps by a proto-Atlantic species, likely resulted from dispersal from the Indian Ocean and transport along the African coastline rather than through the Drake passage, where the water temperatures are far colder than those experienced across the current geographical distribution range of contemporary *Nyctiphanes* species (Rabassa

et al. 2005). Planktic dispersal via the former route has been well-documented in the literature (Haq 1982; Connolly et al. 2003; Gibbons 1995, 1997; Gibbons et al. 1995). Consistent with this hypothesis, *N. capensis* is currently endemic to the Benguela current, which was established approximately 10 Mya (Siesser 1980). In turn, the sister species relationship between *N. capensis* and *N. couchii* suggests colonization of the North Atlantic must have occurred by means of a dispersal event from the southern hemisphere, possibly during a period of glacial cooling and consequent contraction of the tropical regions. Dispersal via this route during the Pleistocene has been well-documented for several marine taxa (Bowen and Grant 1997; Grant and Bowen 1998; Koufopanou et al. 1999; Stepien and Rossenblatt 1996; Stillman and Reeb 2001; Burridge and White 2000; Hilbish et al. 2000; Grant and Leslie 2001). At this time the South Atlantic underwent a period of intensive cooling due to both the reorganization of ocean circulations after the closure of the Central American Seaway and the Pliocene glaciations (Dupont et al. 2005 and references therein).

With the exception of the association between the *N. capensis*–*N. couchii* divergence time and the final closure of the Central American Seaway, the estimated dates of cladogenesis within this genus do not correspond with the other vicariant events documented in the literature (Fig. 2). We therefore conclude founder dispersal rather than vicariance may have played a major role in the generation of the current disjunct geographical distribution among *Nyctiphanes* species.

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