The Dynasty of the Pharaoh: Phylogeography and Cryptic Biodiversity of *Sepia pharaonis* Cuttlefish in Northwest Indian Ocean Peripheral Seas

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Abstract Peripheral seas of the Northwest Indian Ocean and Arabian Sea harbor a huge biodiversity from various taxa including bony and cartilaginous fishes, crustaceans, and mollusks. Many species of cephalopod are also found in this region, including several of appreciable value to fisheries. Despite their economic and ecological importance, substantial taxonomic uncertainty remains for several cephalopod groups in the Northwest Indian Ocean, including squid and cuttlefish. Here we have used currently available genetic data to clarify the cryptic diversity of the *Sepia pharaonis* species complex in the Northwest Indian Ocean and date their lineage divergences. The analyses reveal five distinct lineages, including a Red Sea lineage, an Australian lineage, an Iranian lineage, and central Indian Ocean and western Pacific lineages. The Red Sea clade is consistently recovered as sister to a clade comprising the remaining lineages, with an estimated divergence in the mid-Miocene, approximately 11 million years ago. A similar mid-Miocene divergence in Red Sea pattern has been observed in several marine fish. The number of *S. pharaonis* lineages in the Indo-West Pacific and their divergence times are now quite clear, but the taxonomic status of these lineages and the geological and ecological processes that have influenced their evolution require additional study.

Keywords *Sepia pharaonis* · Phylogeny · NWIO · Peripheral seas

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1 Introduction

The peripheral seas of the Northwest Indian Ocean (NWIO) constitute an economically and ecologically important region of the world ocean. This region includes two of the warmest seas on the planet, which harbor species adapted to high salinity levels and water temperature, oceanographic conditions that are likely to become widespread in the near future due to anthropogenic climate change. In this region, the Red Sea also has been considered as one of the 11 marine biodiversity hotspots (Roberts et al. 2002; Sonnewald and El-Sherbiny 2017) and has a high degree of endemism for fish, coral, and several invertebrate taxa (Bowen et al. 2016). However, more than 40% of global oil production also comes from this region, and oil is a major marine pollutant (Sale et al. 2011) constituting a threat to the regional marine biota. Despite the region's ecological value and the ongoing threats to its biodiversity from oil production and transport as well as climate change, these seas are poorly studied in terms of marine biodiversity. Although there has been considerable research on fish biodiversity globally and in this region, our understanding of the biodiversity of other marine animals remains limited, especially in the northern Indian Ocean. This region is one of the main traditional and artisanal fisheries regions, categorized as fisheries region 51 by the United Nations Food and Agriculture Organization. Several species of northern Indian Ocean cephalopods are targets of artisanal and commercial fisheries (Jereb and Roper 2005, 2010; Jereb et al. 2016), but taxonomic ambiguities and our general lack of understanding of molluscan biodiversity in this region affects fisheries regulation and could lead to a loss of biodiversity in heavily harvested commercial species.

The shallow-water taxon Sepiidae, Leach, 1817 (cuttlefish; Box 1) is a diverse group, comprising over 100 species (Sweeney and Roper 1998) of nektobenthic cephalopod species inhabiting shallow temperate marine waters around islands and in marginal seas (Norman and Reid 2000). Of particular importance to marine fisheries are large-bodied species of the genus *Sepia* Linnaeus, 1758, of which more than 20 species have been described based on morphological and genetic examinations (Table 1), though the existence of several cryptic *Sepia* species (Table 2) has been proposed (e.g., Anderson et al. 2011). *Sepia* is distinguished from the two other sepiid genera by the presence of an elliptical cuttlebone that is approximately as long as the mantle (unlike *Metasepia*) and semicircular mantle-locking apparatuses and lack of a posterior gland and pore (unlike *Sepiella*) (Khromov 1998; Reid et al. 2005a), though a recent phylogenomic study (Lindgren and Anderson 2018) suggests that *Sepia* may be paraphyletic with respect to *Sepiella*. Important morphological characters for species-level identification within *Sepia* include cuttlebone shape, body color pattern, and arm and club sucker patterns (Khromov 1998; Reid et al. 2005a). Unfortunately, these characters often change with growth and may vary between males and females (Reid et al. 2005a).

Table 1 *Sepia* species occurring in the Northwest Indian Ocean (NWIO) with their taxonomic status

Species	Geographic region	Identification method	Last revision (references)	Cryptic diversity/ taxonomic status
S. mirabilis	NWIO	Morphology	Reid et al. (2005a)	No report, data deficiency
S. omani	NWIO	Morphology	Jereb and Roper (2010)	No/unclear
S. pharaonis	IWP	Genetic	This study, Anderson et al. (2007)	Yes/5 clades proposed
S. prashadi	NWIO	Genetic	Reid et al. (2005a)	No/clear
S. ramani	CIO	Genetic	Anderson et al. (2011)	Invalid as distinct species
S. esculenta[a]	IWP	Genetic	Reid et al. (2005b)	No/clear
S. lycidas[a]	WIO	Genetic	Reid et al. (2005b)	No/clear
Sepia aculeata[a]	IWP	Genetics and morphology	Zheng et al. (2004)	No/clear

WIO West Indian Ocean, *NWIO* Northwest Indian Ocean, *IWP* Indo-West Pacific

[a] These species have been used in divergence time estimation for the *S. pharaonis* complex

Table 2 *Sepia* subspecies reported in previous studies

Clade Id	Morphological character	Genetic support	Distribution range	Habitat geographic character[a]
NWIO-ph1	Spotted color in dorsal view of male specimen	COI, 16S, and Rhodopsin	Red Sea	High salinity and temperature, minor exchange with ocean
NWIO-ph2	Spotted color in dorsal view of male specimen	COI and 16S	Persian Gulf and Oman Sea	Higher salinity and temperature
CIO-ph	Broken line color in dorsal view of male specimen	COI	Indian coasts, Thailand southern coasts in Indian ocean	Higher primary production, monsoon
ramani-ph	Long tentacle enlarged medial sucker	No	Sri Lanka and Indian west coasts (Tuticorin bay)	Higher primary production
Au-ph	Male with broken line	COI	Australia	Highly biodiverse
WP-ph	Male with broken line	COI	Taiwan to Thailand	Highly biodiverse

[a] Salinity, primary production, specific current, diversity

Box 1 Classification of the Pharaoh Cuttlefish, *Sepia pharaonis*, an Important Fisheries Species in the Northern Indian Ocean

Phylum: Mollusca, Linnaeus, 1758
    Class: Cephalopoda, Cuvier, 1797 subclass: Coleoidea, Bather, 1888
    Order: Sepiida, Zittle, 1895 suborder: Sepiina
    Family: Sepiidae, Leach, 1817
    Genus: *Sepia*, Linnaeus 1758 subgenus: *Sepia*
    Species: *Sepia pharaonis* Ehrenberg, 1831

Many *Sepia* species have been reported from the coastal regions of the NWIO, especially the Arabian Sea from the west coasts of India and Pakistan to the Oman Sea and Persian Gulf, peripheral to the Red Sea biodiversity hotspot (Anderson et al. 2007; Khatami et al. 2017) (Table 1). Most cuttlefish species in the NWIO are important for local and artisanal fisheries (Jereb and Roper 2005). Cuttlebones from these species are also used for ornamental handicrafts.

Cuttlefish mainly feed on mollusks, young fish, and crabs. *Sepia* species have a relatively short larval period, typically laying eggs one to two times a year depending on temperature in coastal waters, attaching them to seaweeds where they are sheltered from currents (Onsoy and Salman 2003). They have pelagic hatchlings—so-called paralarvae—that mainly feed on zooplankton and probably disperse by currents varying distances, depending on hatchling size and larval duration (Villanueva et al. 2016). Like other cephalopods, cuttlefish are preyed upon by predatory fish and marine mammals.

2 *Sepia pharaonis*

The pharaoh cuttlefish *Sepia pharaonis* Ehrenberg, 1831 is a commercially important fisheries species with a wide geographic distribution (Norman and Reid 2000) ranging from the western Pacific (Japan) to northern Australia and the western Indian Ocean, including the Red Sea, Persian Gulf, and Oman Sea. It is dominant in fisheries catches from several regions including the NWIO and the west Pacific (WP) (e.g., Philippines, Thailand and China) (Reid et al. 2005a) (Fig. 1). This species also has been reared in some regions as an aquaculture candidate due to its high growth rate and adaptability (Barord et al. 2010).

S. pharaonis has been reported to have a high level of cryptic diversity throughout its range. For example, Norman and Lu (2000) noted three distinct forms of *Sepia pharaonis*: one in the NWIO, one in the east Indian Ocean and WP and a third in the central Indian Ocean (CIO), based on morphological differences such as the pattern of lines or spots on the arms. A fourth type might be present in Australian waters (Norman and Lu 2000). Moreover, the three forms noted by Norman and Lu (2000) differ in reproduction season; these differences could have promoted

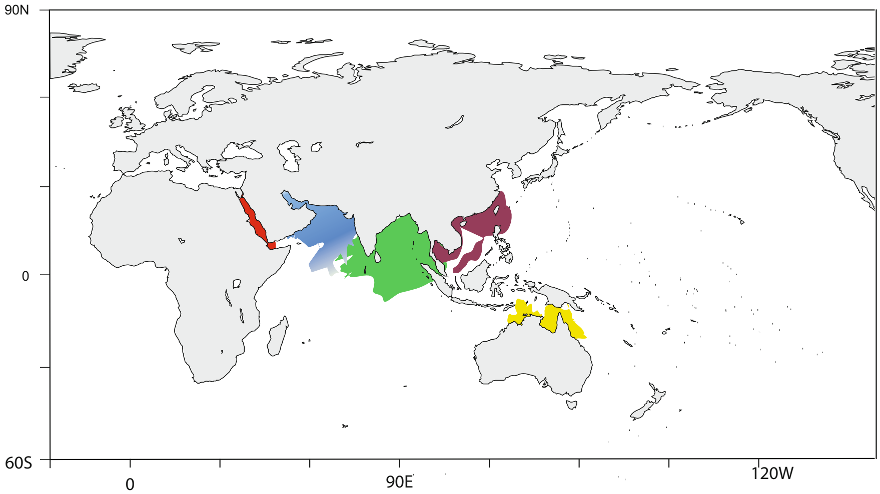

Fig. 1 Distribution map of lineages in the *S. pharaonis* species complex in the Indo-West Pacific. Different colors represent different lineages described in the text—red Red Sea, blue Persian Gulf and Oman Sea (PG &OS), green Central Indian Ocean (CIO), yellow Australia, purple West Pacific (WP)

reproductive isolation (Norman and Reid 2000). An additional form was proposed by Anderson et al. (2007) in the NWIO periphery in the Persian Gulf and Oman Sea based on partial 16S rRNA gene sequences. Anderson et al.'s (2007) analyses also recovered Norman's morphologically described forms. Further investigation using both mitochondrial (16S rRNA and COI) and nuclear (rhodopsin) genes recovered five geographically delimited *S. pharaonis* subclades including Red Sea, Persian Gulf and Oman Sea, CIO, Australian, and WP lineages (Anderson et al. 2011). This study also rejected *Sepia ramani* (Neethiselvan 2001) as a separate species, grouping two specimens identified as *S. ramani* into *S. pharaonis*, one in the CIO *S. pharaonis* lineage and the other as sister to the Australian lineage. A recent investigation of cryptic cephalopod diversity in the Persian Gulf and Oman Sea based on phylogenetic analysis of additional COI and 16S data revealed cryptic cuttlefish diversity in this region and also recovered the Persian Gulf and Oman Sea *S. pharaonis* lineage (Khatami et al. 2017).

Morphological investigation has been proposed for further clarification (Anderson et al. 2011) of the *S. pharaonis* species complex. One difficulty has been the absence of a holotype for *S. pharaonis* for comparison, and the lack of clear and distinct morphological characters that could be used to distinguish the genetic lineages has also been a challenge.

Here we have reconstructed relationships within *S. pharaonis* based on data collected for our previous study and additional new data retrieved from GenBank (Box 2). We have also attempted to estimate dates for the divergences of the *S. pharaonis* lineages using a Bayesian method.

Box 2 Details of Analyses Used to Reconstruct the Phylogenetic Relationships of Pharaoh Cuttlefish Lineages and Estimate Their Times of Divergence

The COI sequences retrieved from GenBank were combined with recently sequenced fragments from *Sepia* collected from the Persian Gulf and Oman Sea and aligned with MAFFT (Katoh et al. 2002) in Geneious (Kearse et al. 2012), and the resulting alignment was adjusted by eye to eliminate misalignments. Bayesian phylogenetic analysis was carried out in BEAST 2 (Bouckaert et al. 2014) with a 50000000-generation MCMC chain and 20% burn-in, with the best-fitting substitution model selected in JmodelTest2 (Darriba et al. 2012) based on BIC (Posada and Buckley 2004).

Species delimitation was implemented using GMYC (Generalized Mixed Yule Coalescent) (Zhang et al. 2013; Fujisawa and Barraclough 2013), PTP (Poisson Tree Processes) (Zhang et al. 2013), and Automatic Barcode Gap Discovery (ABGD) with default setting (priors pmin = 0.001 and pmax = 0.1, with 10 recursive steps and 20 bins, and gap width 1.5) (Puilandre et al. 2012) models using the h-its (http://species.h-its.org/) and ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) web servers. Species delimitation also was carried out in Geneious R9. The outputs of the models were compared and only clades identified based on all three models were flagged as potential cryptic species.

Dating methods: A recently published chronogram (time-tree) for coleoid cephalopods based on analyses of mitochondrial genome data (Uribe et al. 2017) provided the opportunity to estimate divergence times among the *Sepia pharaonis* lineages. Complete COI sequences from mitochondrial genomes for several outgroups represented in the Uribe et al. (2017) tree (*Sepia aculeata*, *Sepia apama*, *Sepia esculenta*, *Sepia latimanus*, *Sepia lycidas*, *Sepia officinalis*, *Sepiella inermis*, and *Sepiella japonica*) were combined with two complete *COI* sequences from representatives of the Red Sea and Central Indian Ocean lineages of *Sepia pharaonis* and partial COI sequences from representatives of the Australian, Persian Gulf, and WP *S. pharaonis* lineages (Table 4).

These data were analyzed in BEAST 2.4.8 (Bouckaert et al. 2014) on CIPRES (Miller et al. 2010) using secondary calibrations taken from Uribe et al. (2017) (Table 4). For each node used for calibration, the 95% highest posterior density interval from Uribe et al. (2017) was approximated with a normal distribution and used as a calibration prior. BEAST analyses were each run twice with different random starting seeds for 50 million generations (sampling every 1000 generations) using Bayesian substitution model averaging (across the 31 models allowing grouping within transitions and within transversions; (Bouckaert and Drummond 2017) with a 20% burn-in. Each analysis used either a fixed topology matching the Uribe et al. (2017) tree and

(continued)

Box 2 (continued)
our ML estimate of relationships among the *S. pharaonis* lineages or simultaneously estimated the tree topology and divergence times (co-estimation resulted in a slightly different topology with respect to relationships among the outgroups than seen in the Uribe and Zardoya study).

For the fixed topology analyses, divergence times were estimated using random local clocks and an uncorrelated lognormal clock, both with default priors. For the co-estimation analysis, only an uncorrelated lognormal clock was used, with default priors as well as diffuse gamma priors (alpha = 0.001; beta = 1000) on the Yule birth rate and clock mean rate (test analyses using random local clocks during co-estimation yielded node age estimates within the *S. pharaonis* complex that were less than one-twentieth as old as those recovered with random local clocks in the fixed topology analysis or either uncorrelated lognormal clock analysis). To determine whether priors were exerting a strong influence on posterior date estimates, we also ran all analyses for 10 million generations sampling only from the prior. To assess convergence for each run, Tracer 1.6 (Rambaut et al. 2014) was used to examine parameter traces and estimated sample sizes.

All species delimitation methods—ABGD (Puilandre et al. 2012), GMYC (Zhang et al. 2013; Fujisawa and Barraclough 2013), PTP (Zhang et al. 2013), and the Geneious 9 (Kearse et al. 2012) species delimitation plug-in (Masters et al. 2011)—verified the five main clades of *S. pharaonis* as distinct species (Fig. 2). The highest Rosenberg P-value was observed for the Australia lineage while the smallest value was for Persian Gulf and Oman Sea clade. The Kimura 2-parameter (Kimura 1980) between- and within-group genetic distances computed in MEGA 7 (Kumar et al. 2016) showed a considerable genetic distance among clades (Table 3). ABGD analysis also showed five groups within the *S. pharaonis* clade (Red Sea, Australian, Iranian coasts of Persian Gulf and Oman Sea, CIO and WP) with prior maximal distance $P = 0.021544$. Geneious species delimitation (clade support of ≥ 90 for all 5 clades) and GYMC analysis returned the same five main groups. The WP clade had highest support (0.9) in GYMC analysis which means it was less likely for WP to be a distinct species from the CIO lineage.

Our current phylogenetic analysis showed three major clades within *S. pharaonis*—one consisted of specimens from the Red Sea, the second clade included Australia samples, while the third clade comprised the rest of the range of *S. pharaonis*, including samples from Persian Gulf and Red Sea to Taiwan. One sample from the Iranian coast (Iran3 = SphOm10) and another from the Oman Sea (SphMuscat) also grouped with the Red Sea lineage, showing a possible range expansion of the Red Sea lineage into other NWIO regions. This type of expansion into the Indian Ocean has been seen in some fish species (DiBattista et al. 2013). The Red Sea has very high biological diversity and some of these taxa have passed through the Suez Canal into the Mediterranean Sea, but to date there has been no

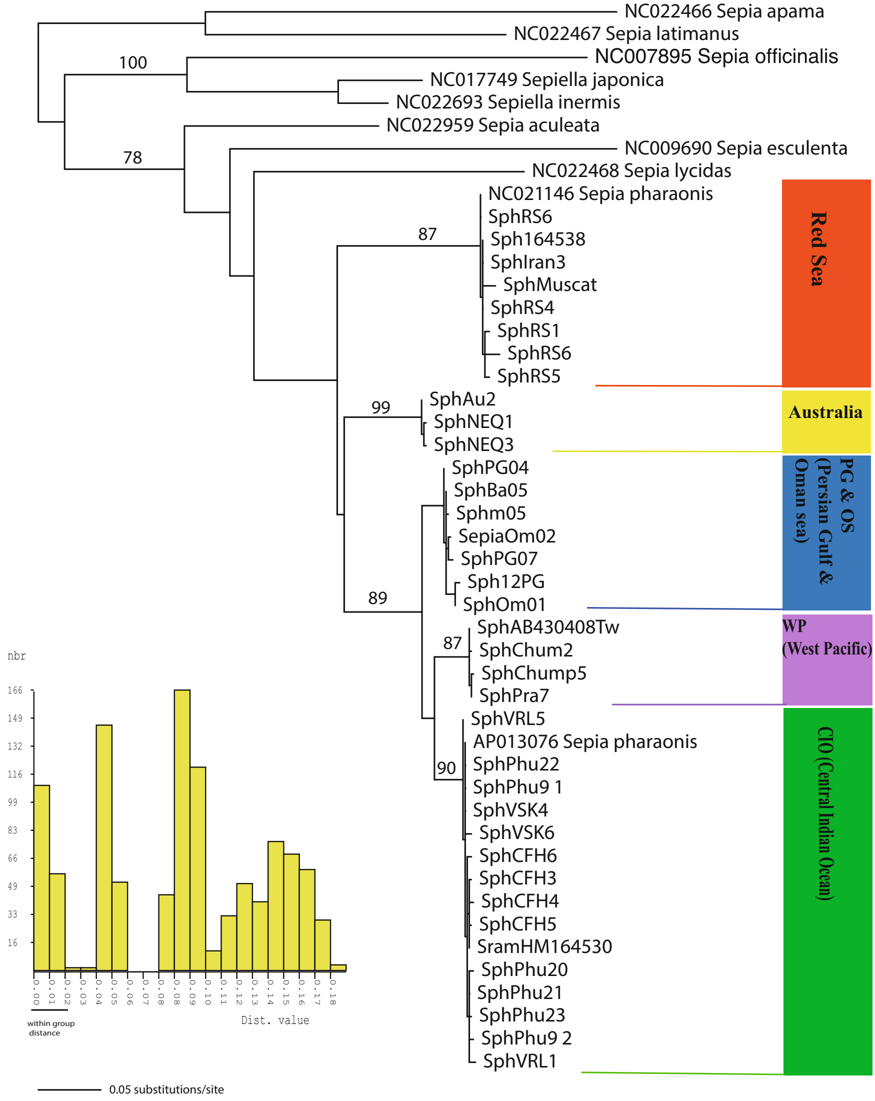

Fig. 2 Phylogenetic relationship among *S. pharaonis* lineages based on currently available genetic data from partial COI sequences. Bayesian posterior probabilities greater than 0.5 and maximum likelihood bootstrap values above 70% are represented on each branch

such report for *S. pharaonis*. The formation of a separate lineage of *S. pharonis* in the Red Sea can be best explained by land-bridge in mid-Miocene within the vicinity of the Red Sea and Gulf of Aden, which reduced the connectivity between this region and the rest of the Indian Ocean (Bosworth et al. 2005). Additionally, the

Table 3 K2P distance among *S. pharaonis* lineages in Indo-Pacific estimated on COI partial sequences

	Red Sea	PG	CIO	Au	Pacific
Red Sea	**0.01 (0.003)**				
PG	0.087	**0.005 (0.002)**			
CIO	0.097	0.045	**0.006 (0.002)**		
Au	0.086	0.086	0.102	**0.002 (0.001)**	
Pacific	0.101	0.048	0.044	0.094	**0.002 (0.002)**

Values in bold represent within-group divergence (SE in parenthesis)

narrow connection between the Red Sea and the Gulf of Aden has always been a potential barrier of gene flow between the Red Sea and rest of NWIO (Obura 2016).

The third clade comprised three subclades—(1) a Persian Gulf and Oman Sea (PG & OS) group, (2) an Indian and Malay Peninsula group (CIO), and (3) a South China Sea (WP) lineage. There was no divergence between samples from Persian Gulf and Oman Sea, suggesting that no physical barrier limits gene flow between the Persian Gulf and Oman Sea. The Persian Gulf is a young biogeographic region formed around 10,000–14,000 years ago after the last glacial maximum (Lambeck 1996), providing relatively little time for genetic divergence to develop between Persian Gulf and Oman Sea populations. Previous studies have also suggested a barrier to gene flow between the Oman Sea and the rest of the NWIO in other species with pelagic larvae (Hoolihan et al. 2004), but the cause of this barrier remains unclear. One possible factor is the Arabian Sea Gyre, which could reduce pelagic larval dispersal or affect food availability and salinity and temperature regimes, limiting movement of marine organisms with low larval dispersal (Villanueva et al. 2016)

3 Divergence Time

Estimating divergence times for cuttlefish is challenging despite the presence of an internal shell (the cuttlebone), because fossil cuttlebones are quite rare. There have been some reports for fossil cuttlebones from the Cenozoic of Europe (Kostak et al. 2016) as well as soft tissue (Košťák et al. 2018) and a cuttlebone identified as *Sepia* from the Miocene (Kostak et al. 2016), but to date, no fossils have been attributed to *S. pharaonis* (Anderson et al. 2011). Uribe et al. (2017) estimated divergence times for most major cephalopod lineages using complete mitochondrial DNA (mtDNA) genomes and several fossils, including those reported in Kostak et al. (2016). Here we have used Uribe and Zardoya's estimates to estimate divergence times within *Sepia* clades and among the *S. pharaonis* lineages (Box 2).

ESSs for all parameters for each divergence time analysis in BEAST were >200, suggesting that all runs converged, and analyses sampling from the prior only yielded date estimates within *S. pharaonis* that were radically different from dates from analyses that incorporated sequence data, suggesting that the calibration priors exert little influence on our estimates within *S. pharaonis*. Most analyses (i.e., fixed topology vs. co-estimation of dates and topology, different clock models and different priors) resulted in similar divergence time estimates for the *S. pharaonis* lineages (Fig. 3). For each analysis, the two independent BEAST runs yielded virtually identical estimates (<1% difference in mean date estimates between runs), so post burn-in trees from each run were pooled. Across all analyses, the Red Sea *S. pharaonis* lineage was estimated to have diverged from the ancestor of the other four lineages approximately 11 million years ago (mean estimates across all three analyses range from 10.95 to 12.31 mya). The Australian lineage diverged from the ancestor of the remaining three lineages 8–10 mya) (range = 8.38–10.33- mya). Divergences between the three remaining lineages are much more recent, between 2 and 4 mya (range = 2.85–3.39 mya). Our divergence time estimates should be considered with caution, because they rely on secondary calibrations from another molecular study.

The mean nucleotide divergence observed among *S. pharaonis* clades was similar species range other for *Sepia* ranged from 6 to 12%. As shown in Table 2, the nucleotide distances among *S. pharaonis* lineages ranged from 4 to 10%. Mean nucleotide divergences within the *S. officinalis* complex were reported to be 5–13% (Healey et al. 2017).

A number of geological events alongside life history and behavioral characteristics of *S. pharaonis* could be implicated to explain the divergence process.

Red Sea: The Red Sea linage divergence around 10–13 million years ago can be best explained by post-Miocene land bridge formation between the Red Sea and Gulf of Aden (10–5.5 mya). In addition, the final closure of the Tethys Sea (the Terminal Tethyan Event, TTE) around 12 mya has been believed to play an important role as a barrier to gene flow in the Arabian Sea region and a source of diversification for several marine taxa including coral reef fishes (Obura 2016), *Stylophora* corals (Arrigoni et al. 2016).

Australia: The Indo-Australian archipelago (IAA) is thought to have developed during the mid-late Miocene, resulting in the diversification of the fauna and flora of the Great Barrier Reef (Obura 2016). During this epoch, the ocean currents and circulations changed in the IAA due to the rise of the Himalayas leading to strengthening of the monsoon current (Lohman et al. 2011); these oceanographic changes could be another possible mechanism explaining the divergence of the Australian *S. pharaonis* clade. For several other species, divergence between Australian lineages and other Indian Ocean lineages has been estimated to occur during the Oligocene (Cowman and Bellwood 2013), somewhat older than our estimate. There are also some evidence of much more recent isolation events during the last glacial maxima, but these also are not in accordance with our estimation. However, our estimate is not unprecedented—there are examples from several marine fish of divergences between IAA lineages and the rest of Indian Ocean at around 6 mya (Cowman and Bellwood 2013). Therefore, it seems that geological events may not have been the sole force of divergence for the Australian *S. pharaonis* clade.

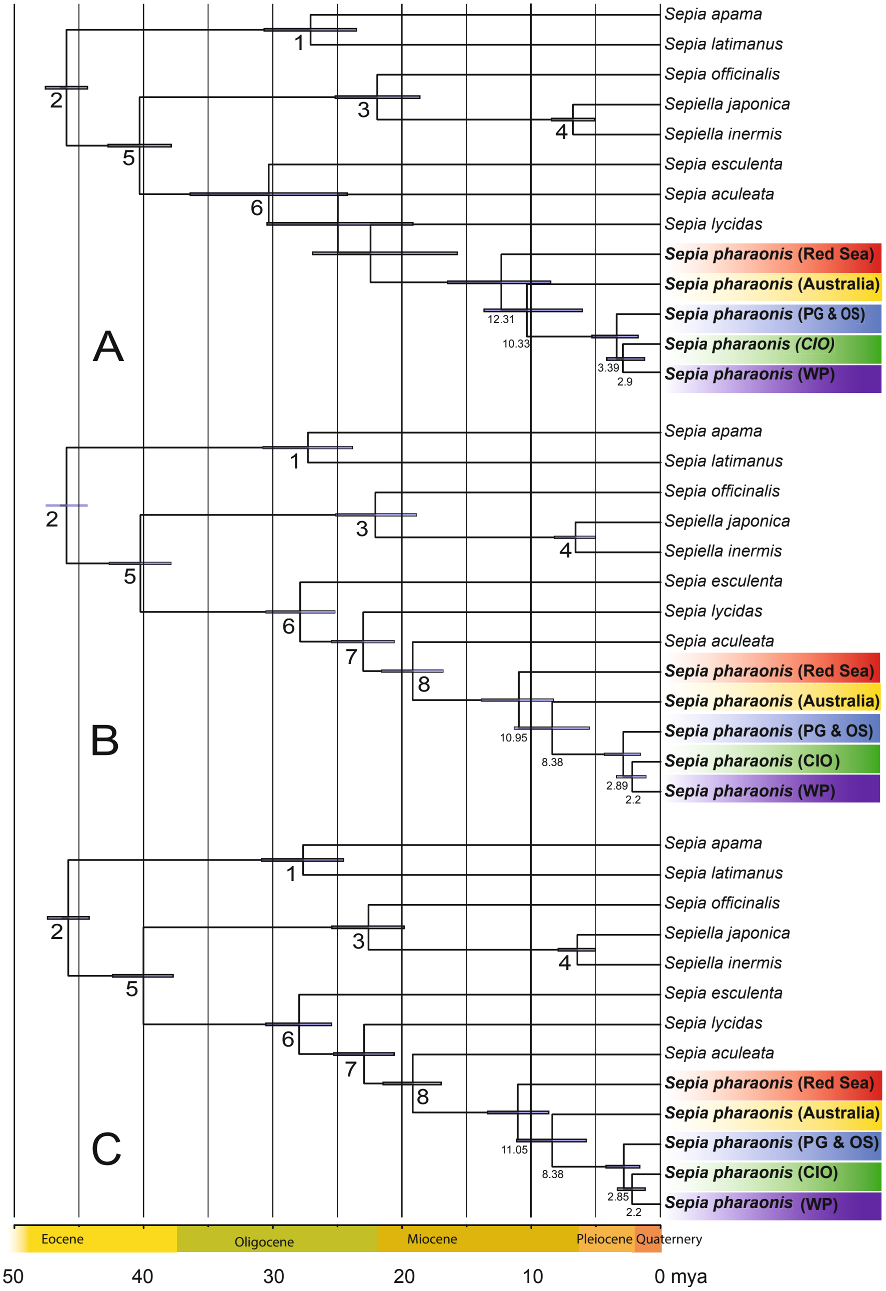

Fig. 3 Chronograms depicting divergence times and 95% highest posterior densities for divergences among the *Sepia pharaonis* lineages. Numbered nodes were used for clock calibration; see Table 4. (**a**) Chronogram based on co-estimation of tree topology and dates, uncorrelated lognormal clock, default priors; (**b**) Chronogram based on BEAST analysis with the tree topology fixed to Uribe et al. (2017) topology, uncorrelated lognormal clock, default priors; (**c**) Chronogram based on BEAST analysis with the tree topology fixed to Uribe et al. (2017) topology, random local clocks, default priors. Accession number for sequence data used in this study

Table 4 Mean, standard deviation and upper and lower 95% quantiles for the normal distributions used for clock calibration and means and 95% upper posterior density date estimates for divergences within the *S. pharaonis* complex

Clade	Co-estimation	U&Z topology uncorrelated lognormal	U&Z topology random local clocks
Red Sea	12.31 (7.87, 15.33)	10.95 (8.28, 13.86)	11.05 (8.65, 13.38)
Australia	10.33 (5.68, 12.63)	8.38 (5.5, 11.32)	8.38 (5.75, 11.13)
Persian Gulf	3.39 (1.59, 4.83)	2.89 (1.57, 4.34)	2.85 (1.61, 4.22)
Central Indian	2.9 (1.15, 3.81)	2.2 (1.13, 3.41)	2.2 (1.19, 3.33)

Node 1 = 25.7 ± 1.98 [21.8, 29.6], Node 2 (root node) = 46.1 ± 0.85 [44.4, 47.8], Node 3 = 19.6 ± 1.8 [16.1, 23.1], Node 4 = 7.1 ± 1.05 [5.04, 9.16], Node 5 = 40.9 ± 1.3 [38.4, 43.4], Node 6 = 27.2 ± 1.65 [24.0, 30.4], Node 7 = 23.58 ± 1.6 [20.4, 26.7], Node 8 = 18.3 ± 1.65 [15.1, 21.5] (nodes 7 and 8 not used in co-estimation analysis due to topological uncertainty)

Persian Gulf and Oman Sea: The establishment of the monsoon current and Arabian Sea Gyre have probably served as a barrier to gene flow since the late Miocene. For example, a study on the sea urchin *Diadema setosum* also shows a divergence between the Arabian Peninsula and the Indo-West Pacific of 3–5 mya (Lessios et al. 2003), supporting our finding in this study. The biogeography of the Arabian Sea region excluding the Red Sea is poorly investigated and further studies are required to clarify the geological and oceanographic processes that may have led to diversification of marine taxa in this region. Similar to our finding for the Persian Gulf and Oman Sea clade, Vogler et al. (2012) proposed a divergence between Oman Sea and central Indian Ocean lineages of the crown-of-thorns starfish of 1.86–2.89 mya, in the late Pliocene-early Pleistocene. Based on anomalous random local clock co-estimation analysis estimates 50,000 years for the divergence of the Persian Gulf lineage, which is more in line with the history/appearance of the Persian Gulf itself. However, the “Persian Gulf lineage” is also found in the Oman Sea, and the divergence of this lineage from the other lineages could have come long before this lineage invaded the Persian Gulf.

Altogether, in addition to geographic barriers, other factors including strong environmental (e.g., salinity) gradients and water mass exchange or changes in currents over geological time and adaptation to different temperatures may explain the divergences we have detected among *S. pharaonis* lineages.

4 Implications for Arabian Sea Fisheries Management and Biodiversity Conservation

There have been several mistakes in fisheries reports due to a failure to recognize cryptic lineages within species. This has led to misconduct in fisheries management and overharvesting of depleted species (Garcia-Vazquez et al. 2012). Although species can be affected by hybridization and other biological events and thus difficult to delineate, the species unit is considered to be of unique management and conservation significance. Clarification of species status in marine taxa could help improve fisheries management and conservation of biodiversity, particularly in regions that are under intense pressure from a variety of human activities. Identification problems at the genus and species levels make landings-based assessment controversial. Molecular barcoding has become a critical tool for the assessment and implementation of urgent management policies for several fish and invertebrate species. Furthermore, fine-scale genetic differences within populations should be investigated to identify stock structures for sustainable fisheries management.

5 Future Directions

Next-generation sequencing (NGS) data have clarified phylogenetic discrepancies across a broad spectrum of marina taxa. NGS methods can yield several hundred to several thousand single-nucleotide polymorphisms (SNPs) covering the nuclear and mitochondrial genomes (McCormack et al. 2013) which can be used for phylogenetic analysis. For example, RAD-sequencing data have been exploited to elucidate the phylogenomic relationships in marine invertebrate taxa such as the sea anemone *Nematostella vectensis* (Reitzel et al. 2013). A recent study by Lindgren and Anderson (2018) demonstrated the utility of transcriptomic data generated with NGS methods for estimating coleoid cephalopod phylogeny. Complete mtDNA genome sequencing has been used in to infer the phylogeny of many marine species, including fish (Miya et al. 2003), crustaceans (Jurado-Rivera et al. 2016), and mollusks (Williams et al. 2014). Whole mitochondrial genome (mitogenome) data also have been used to reveal the phylogenetic position of the Ram's Horn Squid, *Spirula spirula* (Strugnell et al. 2017). Recently mitogenome sequencing has been carried out in *S. aculeata* (Guo et al. 2016) and *S. pharaonis* sampled from the East China Sea. Availability of mitogenome data from other *Sepia* species may help reconstruct phylogenetic relationships and shed light on cryptic diversity.

Additional sampling of males and female specimens from the pharaoh cuttlefish and congener species from the Arabian Sea region, including the coasts of Pakistan, Oman and India, and the other Indo-Pacific oceans for an integrative taxonomic study using genetic (including NGS) and morphological data would greatly clarify cuttlefish diversity in this region and allow for a taxonomic revision of the *S. pharaonis* complex. Additionally, southern distribution range and potential clade of species complex in African coasts such as Madagascar has not been identified and sampling from this periphery is recommended. Samples from Indonesia could resolve the relationship and boundary of Australia and central Indian Ocean *S. pharaonis* clades. Similar investigation in other genera such as loliginid would elucidate the evolutionary drivers of cephalopods in Indo-Pacific.

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