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# Morphometry and morphological phylogeny of *Sepia pharaonis* Ehrenberg, 1831 complex in Thai waters

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**Abstract** Three hundred and fifty-eight specimens of pharaoh cuttlefish, *Sepia pharaonis*, Ehrenberg, 1831 were collected from 30 localities in Thai waters, Gulf of Thailand (Pacific Ocean) and the Andaman Sea (Indian Ocean). Specimens were grouped according to sex, habitats and four categorised types of colour patterns on the dorsal mantle based on the number of stripes on the middle and lateral parts. Morphometry of 70 characters from five character sets of external, cuttlebone, digestive system, reproductive system and hectocotylus morphology were compared. The male and female cuttlefish were significantly different in 38 characters. Four types of males and females were significantly different in 29 and 19 characters. Overall, differences in colour patterns were more prominent in males than in females. Phylogenetic analyses of seven high-weighted characters revealed four likely clades of populations, corresponding to four types of colour patterns on the dorsal mantle.

**Keywords** Morphometry · Morphology · Phylogeny · *Sepia pharaonis* · Thai waters

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## Introduction

*Sepia pharaonis* distributes from the Indian Ocean, including the Red Sea and Arabian Sea, Andaman Sea into the South China Sea, Japan and Northwest Australia in the West Pacific (Reid 1998; Reid et al. 2005). Such a wide distribution raised the question as to whether *S. pharaonis* is actually a single species or a species complex (Khromov et al. 1998; Norman 2000; Anderson et al. 2007, 2011). Norman (2000) suggested that the three forms of *S. pharaonis* differed in morphology, reproductive pattern, spawning season and different distributions range. *Sepia pharaonis* I distributes in the western Indian Ocean from the Red Sea to the Arabian Gulf. Males of this type have zebra lines on the third arm pair during mating. *Sepia pharaonis* II are found from Japan to the Gulf of Thailand, the Philippines and northern Australia. Males have broken lines on the third arm pair. *Sepia pharaonis* III are found from the Maldives to the Andaman Sea coast of Thailand. Males have white spots on the third arm pair. The three forms have different spawning season, during August to October for *S. pharaonis* I an March to May for *S. pharaonis* II. *Sepia pharaonis* III are able to reproduce all year round.

Thailand is located between two oceans, the Andaman Sea, Indian Ocean on the west and the Gulf of Thailand, Pacific Ocean on the east. Chotiyaputta (1982) reported that *S. pharaonis* from the Gulf of Thailand, which might correspond to *S. pharaonis* II of Norman (2000), has two periods of spawning, January to February and July to September. The pharaoh cuttlefish from different localities in Thai waters have different colour patterns, which are observable in the field. Most of specimens could be recognised by their colour patterns whether they are from the Gulf of Thailand or the Andaman Sea. The difference is important in from an aquaculture point of view. *Sepia pharaonis* from the Andaman Sea populations was reported to grow faster than those from the Gulf of Thailand in captivity (Nabhitabhata and Nilaphat 1999). The external morphometric study suggested that they were from different populations (Tuanapaya and Nabhitabhata 2016) and molecular analysis supported the morphometric study (Tuanapaya and Nabhitabhata 2014). The aim of this study is to investigate whether there are any morphological variations (based on colour patterns) in this 'complex' and to determine the levels of such variations (intra- or interspecific), as well as phylogenetic relationships among those variations.

## Materials and methods

Mature specimens of *S. pharaonis* were collected from 30 localities (Fig. 1) along Peninsular Thailand; 92 from the Gulf of Thailand and 266 from the Andaman Sea. For the morphological study, specimens of pharaoh cuttlefish were categorised into four types (Fig. 2) based on colour patterns on their dorsal mantle. Type 1, 50% of the dorsal mantle has 6–8 stripes on the lateral side and 18–23 stripes on the middle part. Type 2, 50% of the dorsal mantle is similar to type 1, with 6–8 stripes on the lateral side but 32–36 stripes on the middle part. Type 3, 50% of the dorsal mantle has 13–16 stripes on the lateral part of the dorsal mantle and 18–23 stripes on the middle part. Type 4 is similar to type 3 with 13–16 stripes on the lateral part but with 32–36 stripes on the middle part. The collected specimens comprised 75 males and 33 females of type 1, 39 males and 50 females of type 2 and 11 males and 7 females of type 3. All these specimens of types 1–3 were collected only from the Andaman Sea and could not be collected from the Gulf of Thailand in the present study. Type 4 includes 27 males and 31 females from the Andaman Sea and 44 males and 45 females from the Gulf of Thailand.

Morphometrical data of specimens were obtained from counts and measurements transformed as character indices (percentage of mantle length) for 70 characters in five datasets (Appendix Tables 1, 2, 3, 4 and 5), including external morphometry (37 characters), cuttlebone morphometry (eight characters), digestive system morphometry (nine characters), reproductive system morphometry (11 characters) and hectocotylus morphometry (five characters). The definition of characters and their abbreviations followed Jereb et al. (2005), Reid et al. (2005), Dunning et al. (1998) and Roper and Voss (1983).

Data were grouped according to their types of colour patterns (types 1–4) and sex (male and female). Statistical analyses were undertaken using the SPSS software package, version 11.5. Discriminant analyses (DA) were performed to determine significant differences among group characters in each dataset. Characters with significant differences from each dataset were weighted to select significant individual characters (of each dataset) for phylogenetic analysis by one-way analysis of variance (ANOVA) with least significant difference (LSD) test.

In order to avoid forced (phylogenetic) analysis (via grouping of types and sex), the types of colour patterns were included as one character with four states (types 1–4). Sex was also included as one character with two states. Weighted characters were arranged by the DELTA (DEscription Language for TAxonomy) software (Dallwitz et al. 1993). The consensus phylogenetic trees were constructed using PAUP* software, version 4.0 (Swofford 2002) with the maximum parsimony and the confidence levels were tested by bootstrapping. The spineless cuttlefish, *Sepiella inermis* (Van Hasselt, 1835), was used as an outgroup.

## Results

### External morphometry

In the overall comparison among eight groups (four types, two sexes), males and females were significantly different. Males of types 1 and 3 were different from the other groups in 27 (from 37) characters ($P < 0.05$); arm I–IV length (AL1, AL2, AL3, AL4), web A–E depth (WDA, WDB, WDC, WDD, WDE), tentacle length (TTL), club length (CL), fin length (FL), fin width (FW), number of enlarged suckers on the left and right tentacular clubs (CSeL, CSeR), number of greatly enlarged suckers on the left and right tentacular clubs (CSgeL, CSgeR), club suckers series left and right tentacular arms (CSSL, CSSR), number of suckers on the right tentacular club (CSR), diameter of normal, enlarged and greatly enlarged suckers on the left (CSDnL, CSDeL, CSDgeL) and right tentacular clubs (CSDnR, CSDeR, CSDgeR) and body weight (W). Males of types 2 and 4 and females of all four types were not significantly different (Wilks' $\lambda = 0.66$, $\chi^2 = 398.45$) (Fig. 3a).

In a separate comparison of each sex (four types, one sex), males of all four types were significantly different ($P < 0.05$) in 18 characters; mantle width (MW), AL1, AL2, AL3, AL4, WDA, WDB, WDC, WDD, WDE, TTL, CSeL, CSeR, CSgeL, CSgeR, CSSL, CSSR and CSR) (Wilks' $\lambda = 0.18$, $\chi^2 = 125.18$) (Fig. 3b). Females of all four types (four types, one sex) were also significantly different in a similar manner to males ($P < 0.05$) but only in 12 characters [WDA, WDB, WDC, WDD, funnel length (FNL), CSgeL, CSDnL, CSDeL,

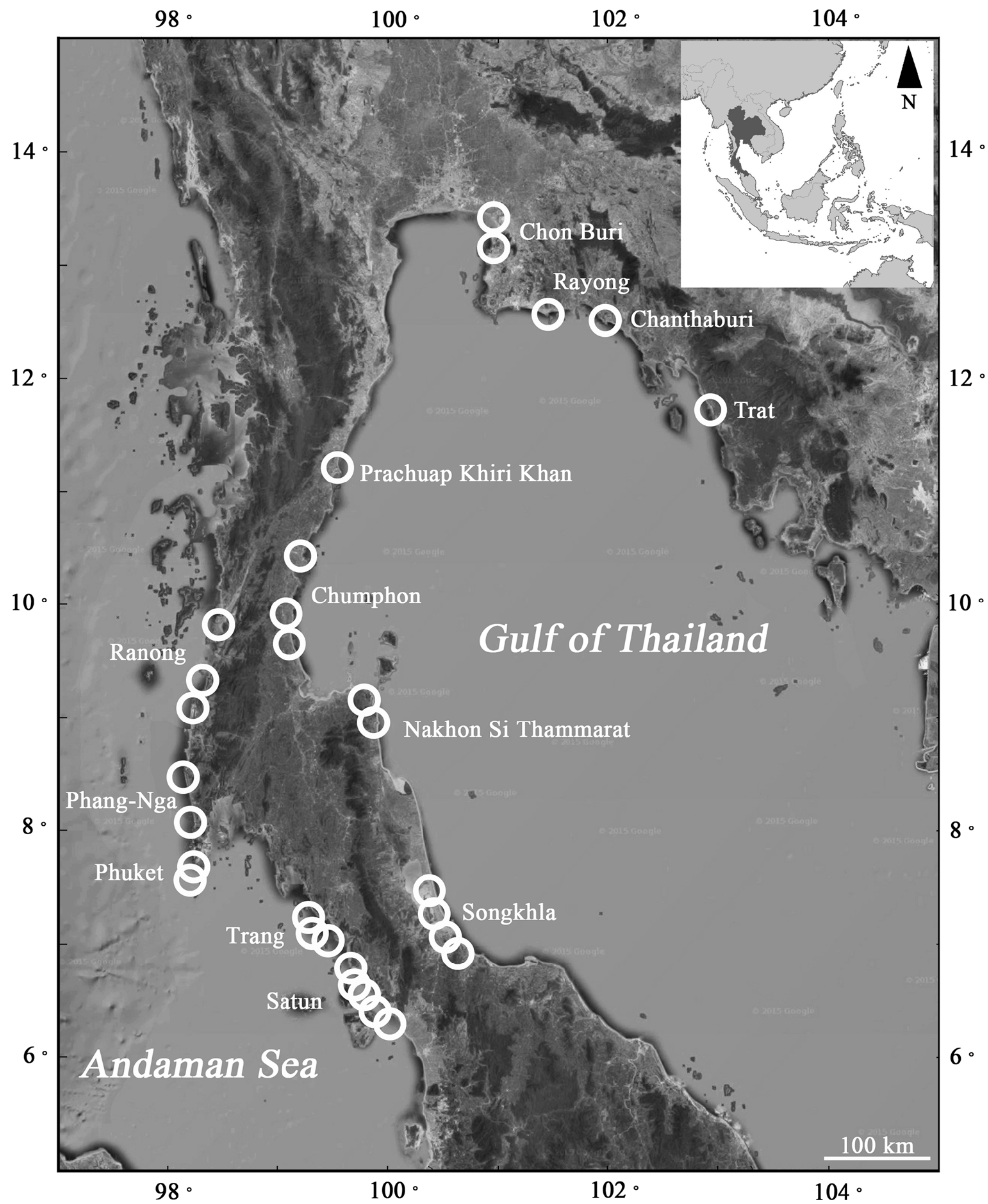


**Fig. 1** Localities of *Sepia pharaonis* specimen collection in Thai waters

CSDgeL, CSDnR, CSDeR and CSDgeR] (Wilks' $\lambda = 0.51$, $\chi^2 = 55.92$) (Fig. 3c).

### Cuttlebone morphometry

In the overall comparison (four types, two sexes), females of type 3 were significantly different ($P < 0.05$) from the other types in six (from eight) characters; including cuttlebone length (CBL), cuttlebone width (CBW), last loculus length (LLL), striated zone length (STL), inner cone width (ICW) and outer cone width (OCW) (Wilks' $\lambda = 0.50$, $\chi^2 = 234.02$) (Fig. 4a).

Males of all four types (four types, one sex) were significantly different ($P < 0.05$) in three characters, CBW, LLL and OCW (Wilks' $\lambda = 0.85$, $\chi^2 = 28.22$) (Fig. 4b). Among females (four types, one sex), females of type 3 were significantly different from the other types ($P < 0.05$) in two characters, CBW and STL (Wilks' $\lambda = 0.90$, $\chi^2 = 18.94$) (Fig. 4c).

### Digestive system morphometry

In the overall comparison (four types, two sexes), males of type 1 and females of type 3 were significantly different from the other types ($P < 0.05$) in five (from 12) characters; stomach width (SW), oesophagus width (EW), digestive gland length (DGLr), digestive gland width (DGWl) and ink sac length (ISL) (Wilks' $\lambda = 0.74$, $\chi^2 = 101.63$) (Fig. 5a).

Males of types 2 and 4 (four types, one sex) were significantly different ($P < 0.05$) from types 1 and 3 in three characters, DGLr, DGWl and ISL (Wilks' $\lambda = 0.88$, $\chi2 = 20.82$) (Fig. 5b). Females of types 3 and 4 (four types, one sex) were

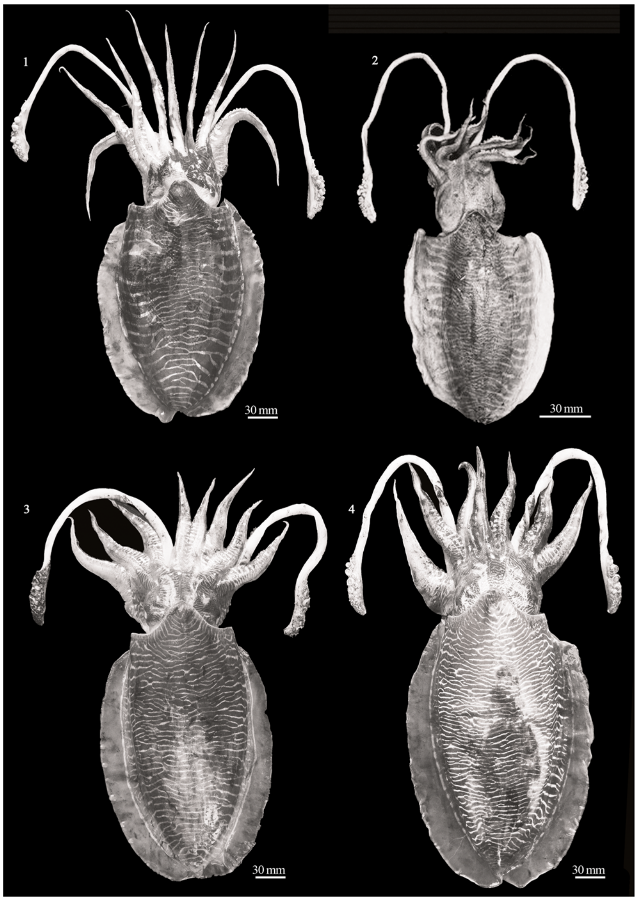


**Fig. 2** The four colour patterns of *Sepia pharaonis* in Thai waters, types 1 to 4, respectively

significantly different ($P < 0.05$) from types 1 and 2 in three characters, oesophagus width (EW), DGWl and CCW (Wilks' $\lambda = 0.86$, $\chi 2 = 25.51$) (Fig. 5c).

### Reproductive system morphometry

Reproductive system characters of males and females were not significantly different in either the overall analysis (four types, two sex) or separate analysis (four types, on sex) (Fig. 6a, b).

### Hectocotylus morphometry

Three (from five) characters, hectocotylised arm length (HAL), hectocotylus length (HcL) and number of modified-sucker series (MSS), were significantly different ($P < 0.05$) among males of all four types (Wilks' $\lambda = 0.73$, $\chi^2 = 53.08$) (Fig. 6c).

### Combined analysis of morphometry

In the comparison of eight groups (four types, two sexes), males and females of all four types were significantly different ($P < 0.05$) in 38 characters; AL1, AL2, AL3, AL4, WDA, WDB, WDC, WDD, WDE, FL, FW, FNL, TTL, CL, W, CSeL, CSeR, CSgeL, CSgeR, CSDnL, CSDeL, CSDgeL, CSDnR, CSDeR, CSDgeR, CSL, CSR, CSSL, CSSR, CBL, CBW, LLL, STL, SL, SW, CCL, CCW and ISL (Wilks' $\lambda = 0.11$, $\chi^2 = 248.40$) (Fig. 7a).

Males of all four types (four types, one sex) were significantly different ($P < 0.05$) in 29 characters; AL1, AL2, AL3, AL4, WDA, WDB, WDD, WDE, FFL, TTL, W, CSeL, CSeR, CSgeL, CSgeR, CSDgeL, CSDgeR, CSL, CSR, CSSR, LLL, STL, DGLr, DGWl, SpC, HAL, HcL, DSS and W (Wilks' $\lambda = 0.005$, $\chi^2 = 168.85$) (Fig. 7b). Females of all four types (four types, one sex) were also significantly different ($P < 0.05$) in 19 characters; WDA, WDB, WDC, WDD, WDE, FL, FNL, CSgeL, CSDnL, CSDeL, CSDgrL, CSDnR, CSDeR, CSDgeR, STL, EW, ISL, OL and OW (Wilks' $\lambda = 0.002$, $\chi^2 = 257.03$) (Fig. 7c).

### Weighted characters

Six characters had significant differences or were weighted, comprising two characters from external morphometry datasets (AL1 and AL4), one from cuttlebone datasets (LLL), one from digestive system datasets (SL), one from reproductive system morphometry datasets (SSL) and one from hectocotylus datasets (HcL). Character states were categorised based on their significant differences and coded as follows:

Arm I length (AL1) character states: 0 = >50% of DML; 1 = ≤50% of DML
Arm IV length (AL4) character states: 0 = ≥70% of DML; 1 = <70% of DML
Last loculus length (LLL) character states: 0 = ≥25% of DML; 1 = <25% of DML
Stomach length (SL) character states: 0 = >20% of DML; 1 = ≤20% of DML
Spermatophoric sac length (SSL) character states: 0 = >40% of DML; 1 = ≤40% of DML
Hectocotylus length (HcL) character states: 0 = >40% of DML; 1 = ≤40% of DML

Characters of colour patterns and sex were coded as follows:

Colour patterns character states: 0 = type 1; 1 = type 2; 2 = type 3; 3 = type 4
Sex character states: 0 = male; 1 = female

### Morphological phylogeny

The phylogenetic tree comprised two major clades (tree length = 124, CI = 0.8629 and RI = 0.9097) (Fig. 8). The first

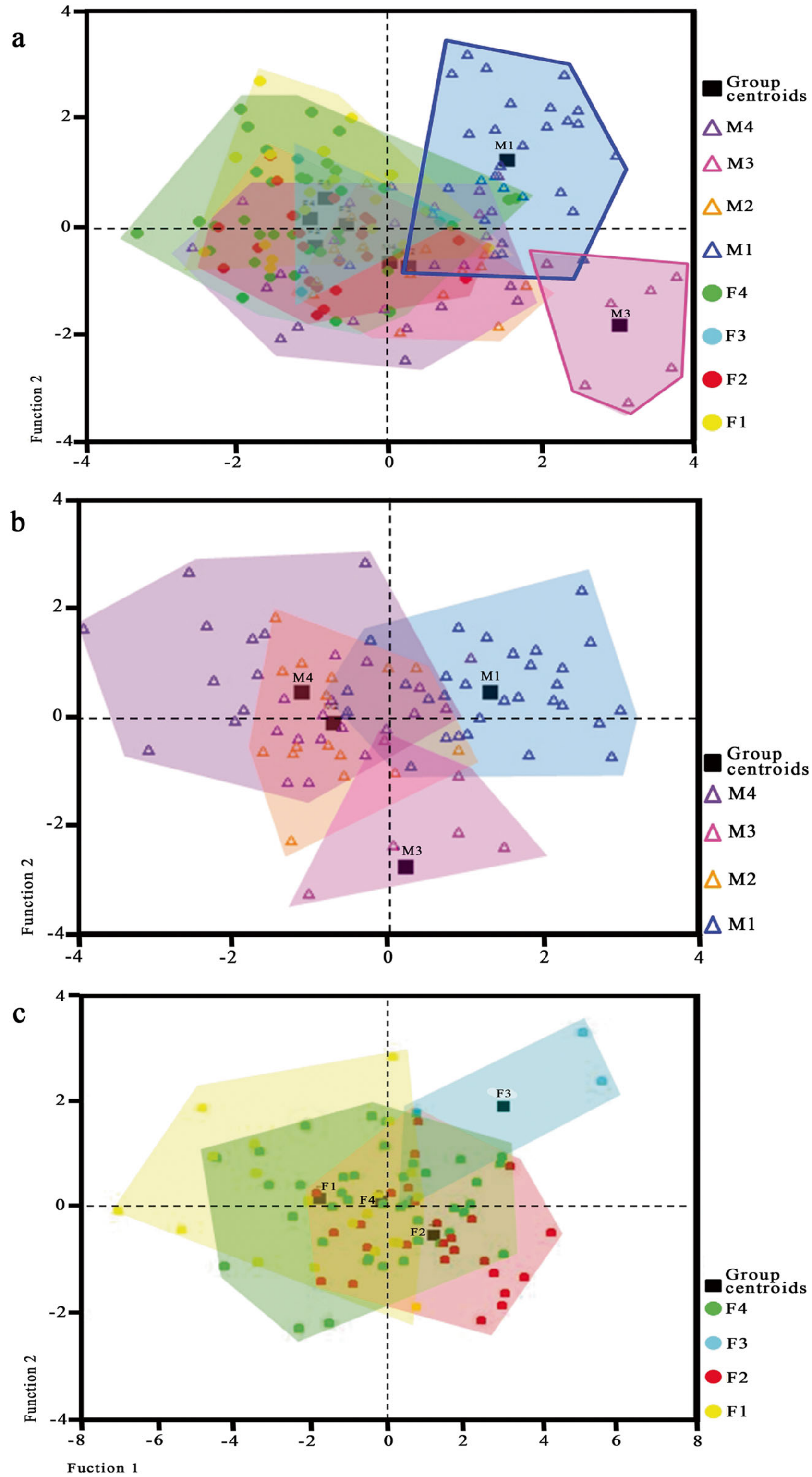


**Fig. 3** Scatter plot of results from external morphometric analysis of *Sepia pharaonis* samples from Thai waters: **a** both sexes, **b** males and **c** females. The analysis includes males of type 1 (*M1*, blue triangles), type 2 (*M2*, orange triangles), type 3 (*M3*, pink triangles) and type 4 (*M4*, purple triangles), and females of type 1 (*F1*, yellow circles), type 2 (*F2*, red circles), type 3 (*F3*, blue circles) and type 4 (*F4*, green circles)

clade is the clade of cuttlefish of type 4 only from both the Gulf of Thailand and the Andaman Sea, but mostly from the Gulf of Thailand (BS = 70.58). The second clade consisted of six subclades. Subclades 1 and 2 were cuttlefish of type 4 (BS = 70.09 and 63.42) and subclade 3 was of type 3 (BS = 59.01). The subclade 4 included the cuttlefish of all four types (BS = 75.14). Subclades 5 and 6 included only cuttlefish of types 1–3 from the Andaman Sea (BS = 65.05 and 50.94).

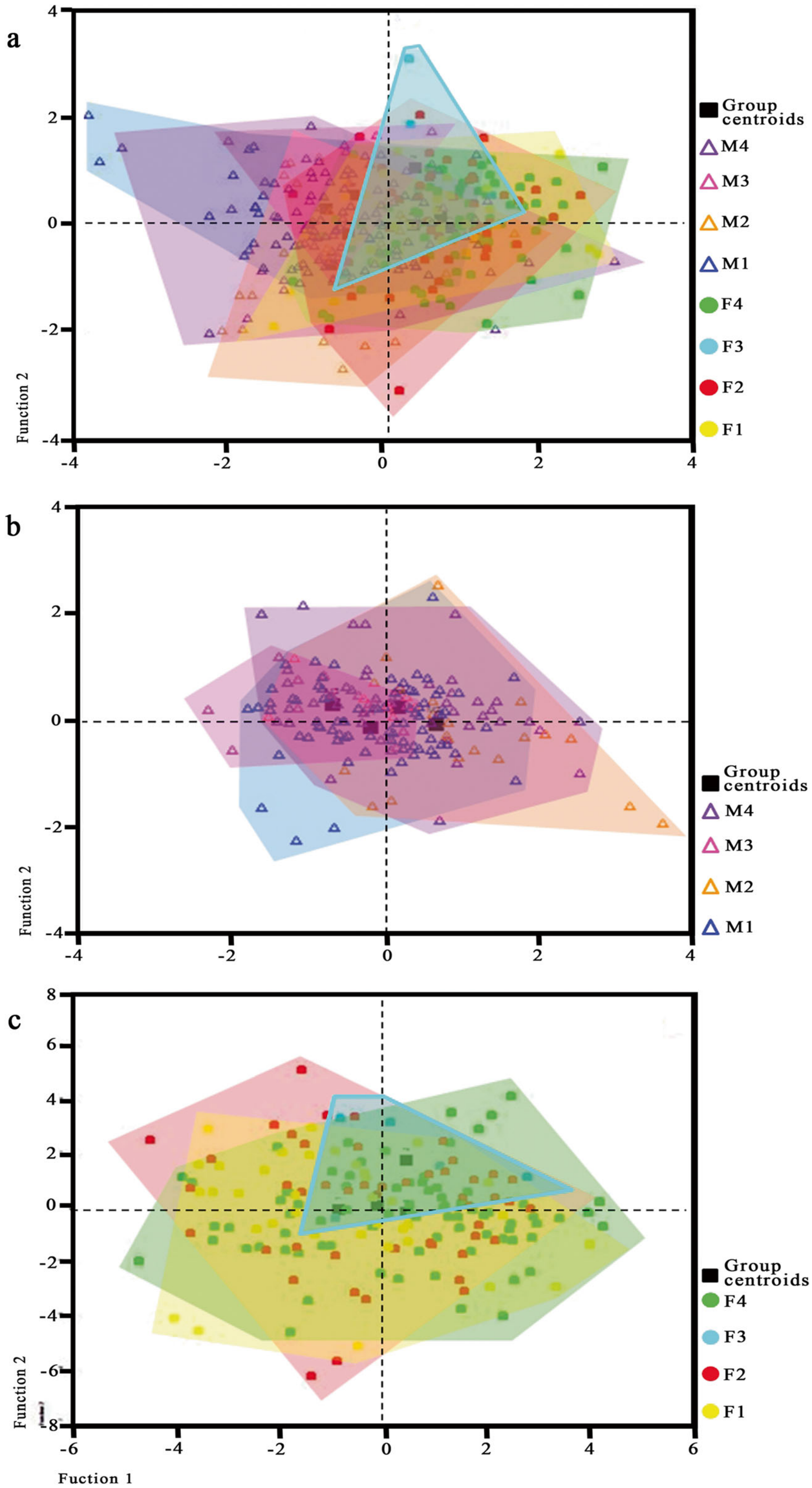


**Fig. 4** Scatter plot of results from cuttlebone morphometric analysis of *Sepia pharaonis* samples from Thai waters: **a** both sexes, **b** males and **c** females. The analysis includes males of type 1 (*M1*, blue triangles), type 2 (*M2*, orange triangles), type 3 (*M3*, pink triangles) and type 4 (*M4*, purple triangles), and females of type 1 (*F1*, yellow circles), type 2 (*F2*, red circles), type 3 (*F3*, blue circles) and type 4 (*F4*, green circles)

## Discussion

The differences in colour patterns of cuttlefish, including *S. pharaonis*, were more prominent in males than in females (Tehranifard and Dastan 2011; Nabhitabhata and Nilaphat 1999). The sexual dimorphisms also exhibited significant differences in arm length I–IV and hectocotylus characters in males and size of tentacular club sucker in females in this study, which agreed with Tuanapaya and Nabhitabhata (2016), who reported that the arm length IV of the pharaoh

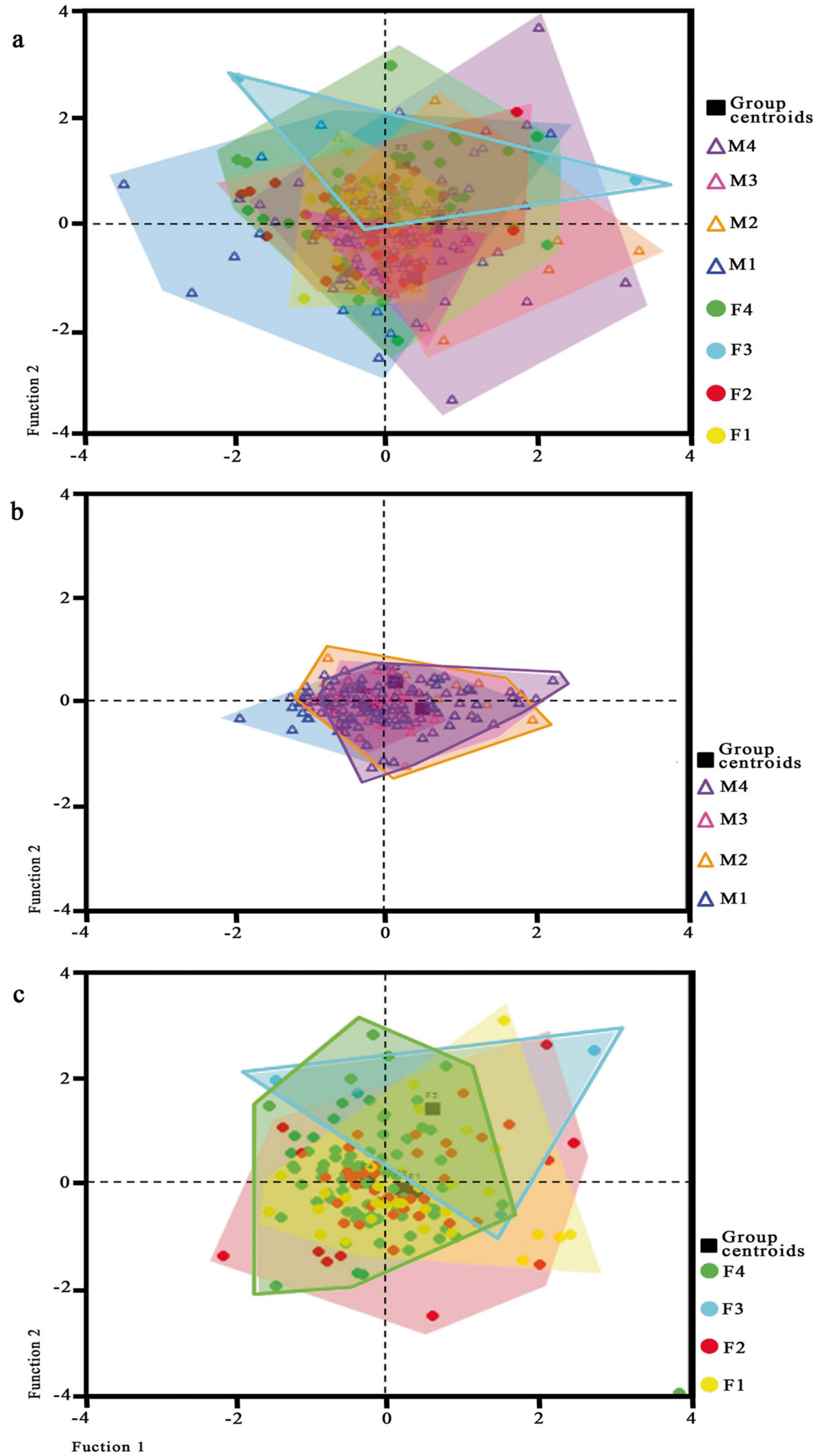


**Fig. 5** Scatter plot of results from digestive system morphometric analysis of *Sepia pharaonis* samples from Thai waters: **a** both sexes, **b** males and **c** females. The analysis includes males of type 1 (*M1*, blue triangles), type 2 (*M2*, orange triangles), type 3 (*M3*, pink triangles) and type 4 (*M4*, purple triangles), and females of type 1 (*F1*, yellow circles), type 2 (*F2*, red circles), type 3 (*F3*, blue circles) and type 4 (*F4*, green circles)

cuttlefish from the Andaman Sea and the Gulf of Thailand were significantly different, suggesting different populations. The differences in hectocotylus of *S. pharaonis* from different geographies, Japan and Australia, had also been previously suggested by Reid et al. (2005).

Differences in cuttlebone morphometric characters were prominent in males. These differences in characters of cuttlebone are one of the best for interspecific identification (Lu 1998; Nesis 1987). The differences revealed in the present study might be used for interspecific identification (of different morphs) in the

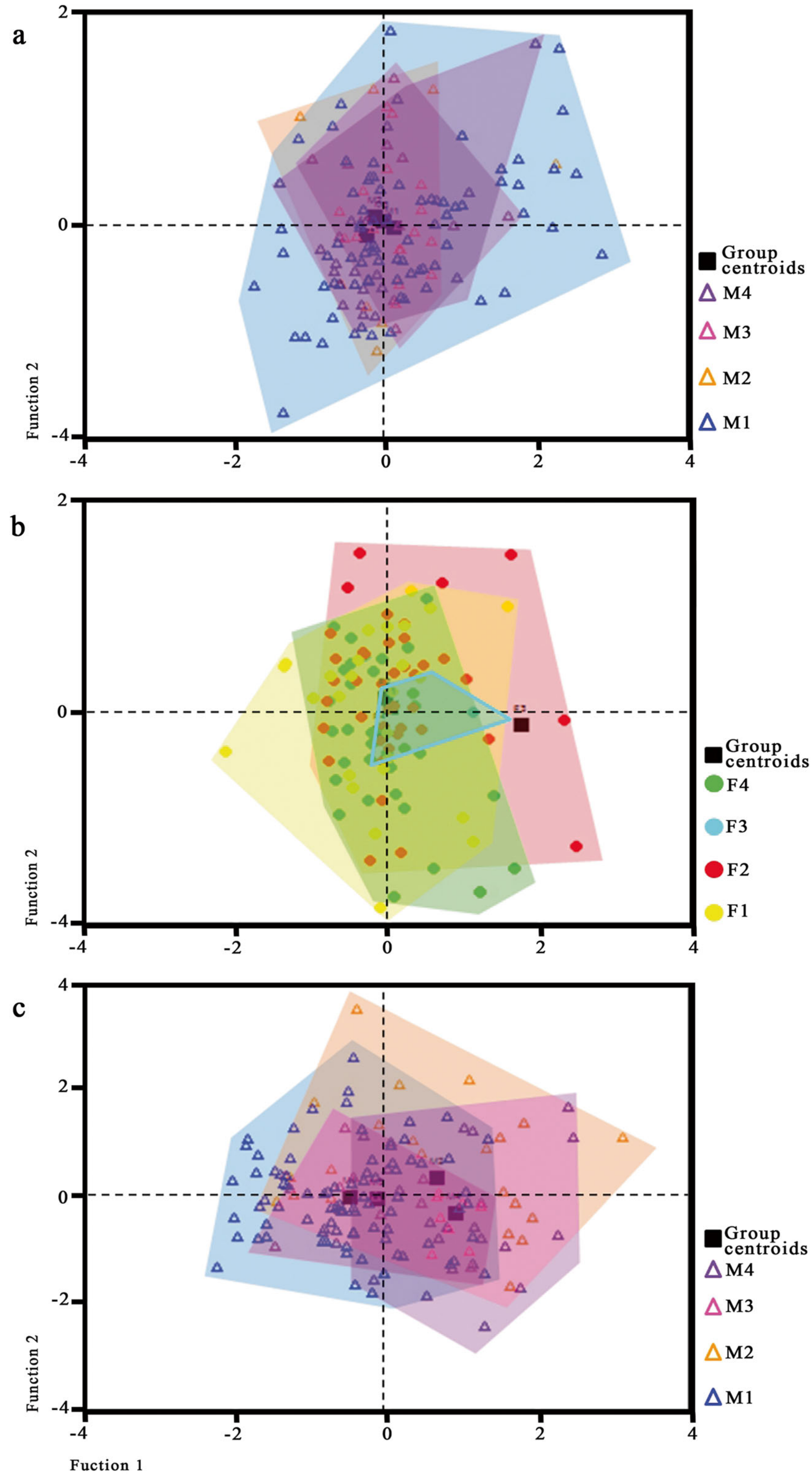


**Fig. 6** Scatter plot of results from reproductive system morphometric analysis (**a** males, **b** females) and hectocotylus morphometric analysis (**c**) of *Sepia pharaonis* samples from Thai waters. The analysis includes males of type 1 (*M1*, blue triangles), type 2 (*M2*, orange triangles), type 3 (*M3*, pink triangles) and type 4 (*M4*, purple triangles), and females of type 1 (*F1*, yellow circles), type 2 (*F2*, red circles), type 3 (*F3*, blue circles) and type 4 (*F4*, green circles)

species complex. On the other hand, variations of cuttlebone characters in *Sepia* might be geographic, as stated by Bonnaud et al. (2006), and types of *S. pharaonis* in this study differ in appearance as they originate from different waters.

Differences in digestive system characters depended on food or types of prey or the process of food storage (Mangold and Young 1998). The differences in the digestive system revealed in the present study might be due to

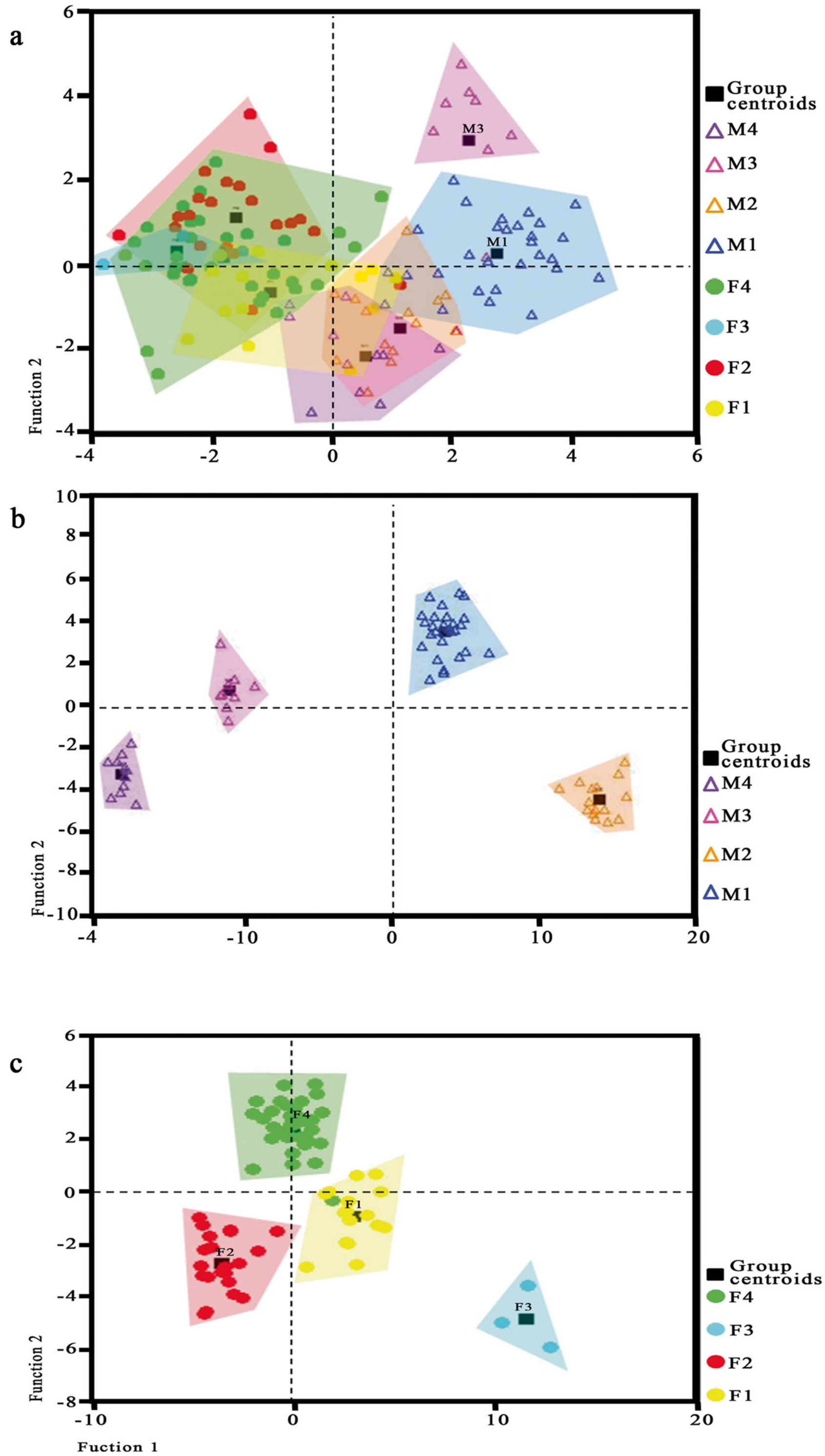


**Fig. 7** Scatter plot of results from the combined morphometric analysis of *Sepia pharaonis* samples from in Thai waters: **a** both sexes, **b** males and **c** females. The analysis includes males of type 1 (*M1*, blue triangles), type 2 (*M2*, orange triangles), type 3 (*M3*, pink triangles) and type 4 (*M4*, purple triangles), and females of type 1 (*F1*, yellow circles), type 2 (*F2*, red circles), type 3 (*F3*, blue circles) and type 4 (*F4*, green circles)

similar causes, since they are from two different habitats and, subsequently, belonged to different populations.

Characters of the reproductive systems of either males or females in this study were different without significance, probably due to insufficient numbers of studied characters. Other characters, especially spermatophore characters (length and shape of different components), should be added in further studies. The spermatophore characters were not determined in detail in the present study, as specimens were in poor condition because of unattended storage and transportation, either onboard or at landing.

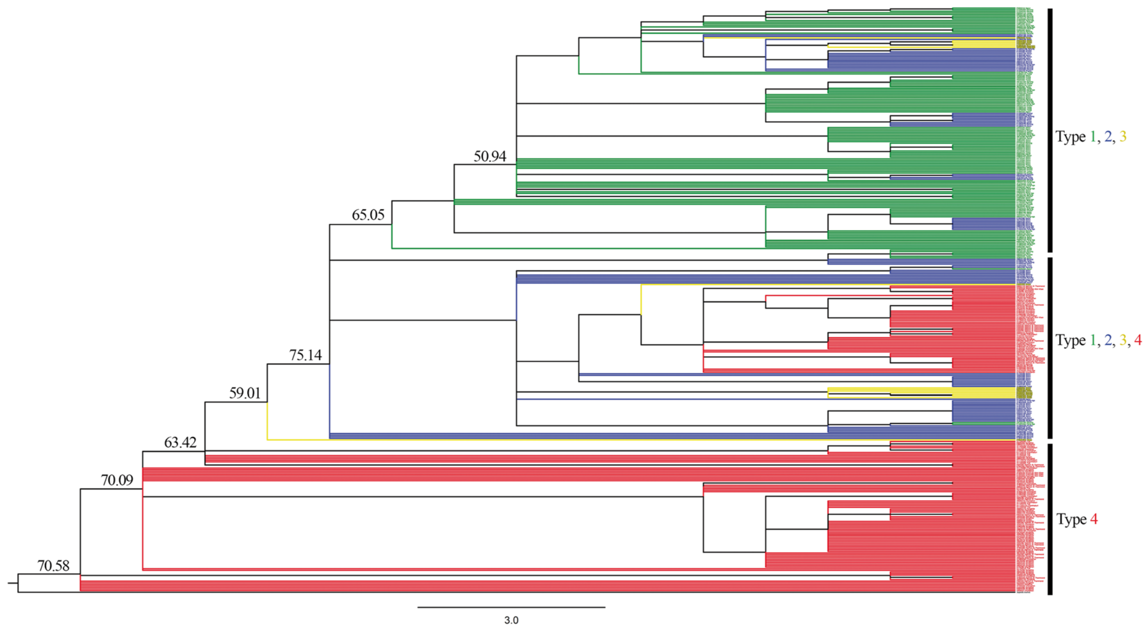


**Fig. 8** The combined morphological phylogeny of four types of *Sepia pharaonis* (maximum parsimony; tree length = 9, CI = 0.6667 and RI = 0.9890), type 1 in green, type 2 in blue, type 3 in yellow and type 4 in red; the numbers are bootstrap support values (BP)

The occurrence patterns of *S. pharaonis* in this study agreed with either morphological observation (Norman 2000) or molecular studies (Anderson et al. 2007, 2011; Tuanapaya and Nabhitabhata 2014). The morphology of pharaoh cuttlefish of type 4 in this study corresponded to *S. pharaonis* II (Japan to the Gulf of Thailand, the Philippines and northern Australia) and types 1–3 corresponded to *S. pharaonis* III (the Maldives to the Andaman Sea coast of Thailand) of Norman (2000). Comparing to molecular studies (16S and COI mitochondrial genes and rhodopsin gene), the occurrence of *S. pharaonis* type 4 in this study corresponded to that of group 4 of Anderson et al. (2007, 2011) in the Western Pacific (the Gulf of Thailand and Taiwan to northern Australia). Types 1–3 corresponded to the occurrence of group 3 (Indian Ocean and the Andaman Sea) (Anderson et al. 2007, 2011).

The first and strongly supported clade of the tree consisted of cuttlefish of type 4, which were mostly specimens from the Gulf of Thailand, supported by its occurrence, as discussed above, made this clade likely to be a geographical one. However, some specimens of type 4 included in this clade were from the Andaman Sea. Such results indicate the tendency that the cuttlefish of type 4 in the Gulf of Thailand belongs to different populations. On the other hand, type 4 and the other three types from the Andaman Sea were included together in the second clade with several subclades, although not strongly bootstrap supported (BS < 70%). Khromov et al. (1998) suggested that the genus *Sepia* originated in the Mediterranean region during the Late Eocene (more than 30 mya: Košťák et al. 2013) and dispersed through the shallow seaway into the present Indian Ocean. Khromov et al. (1998) stated that the Indo-Pacific region is the most important centre of speciation of the family Sepiidae. The mixture of occurrence of all four types in the Andaman Sea and the major occurrence of type 4 in the Gulf of Thailand might indicate that *S. pharaonis* populations in the Andaman Sea are intermediate populations (a gene pool) between populations (of types 1–4) in the Andaman Sea and in the Gulf of Thailand. Based on such an assumption, it might be interpreted that the route of dispersal of *S. pharaonis* is from the Andaman Sea (types 1–4) into the Gulf of Thailand (mainly by type 4) and the West Pacific, and, consequently, allopatric variation might occur in the future.

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## Appendix

**Table 1** External morphometric characters of *Sepia pharaonis* Ehrenberg, 1831 used in this study (modified from Jereb et al. (2005) and Roper and Voss (1983))

| Character | Abbreviation | Definition |
|---|---|---|
| Dorsal mantle length | DML | Dorsal mantle length from anterior most point of mantle to posterior apex of mantle |
| Ventral mantle length | VML | Ventral mantle length from anterior most point of mantle to posterior apex of mantle |
| Mantle width | MW | Greatest straight-line (ventral) width of mantle |
| Fin length | FL | Length of fins from anterior margin of fins to the posterior extreme of mantle |
| Fin width | FW | Greatest width (ventral) of fins between their lateral margins |
| Funnel length | FNL | Length of funnel from anterior funnel opening to posterior border |
| Free funnel length | FFL | Length of funnel from the anterior funnel opening to the point of dorsal attachment to the head |
| Head length | HL | Dorsal length of head from point of fusion of dorsal arm to anterior tip of nuchal locking cartilage |
| Head width | HW | Greatest width of head at level of eyes |
| Arm length | AL1 | Length of arm from first basal sucker to tip of dorsal arm |
| Arm length | AL2 | Length of arm from first basal sucker to tip of dorso-lateral arm |
| Arm length | AL3 | Length of arm from first basal sucker to tip of ventro-lateral arm |
| Arm length | AL4 | Length of arm from first basal sucker to tip of ventral arm |
| Tentacle length | TTL | Length of the right tentacle from junction of arms III and IV to distal extreme |
| Club length | CL | Length of club from proximal most carpal sucker to distal tip of club |
| Web depth | WDA | Distance from mouth to shallowest point of web between adjacent dorsal arms |
| Web depth | WDB | Distance from mouth to shallowest point of web between adjacent of dorsal and dorso-lateral arm |
| Web depth | WDC | Distance from mouth to shallowest point of web between adjacent of dorso-lateral and ventro-lateral arm |
| Web depth | WDD | Distance from mouth to shallowest point of web between adjacent of ventro-lateral and ventral arm |
| Web depth | WDE | Distance from mouth to shallowest point of web between adjacent ventral arm |
| Eye diameter | ED | Diameter of eye across bulbous |
| Club sucker enlarged left | CSeL | Number of enlarged suckers on left tentacular club |
| Club sucker enlarged right | CSeR | Number of enlarged suckers on right tentacular club |
| Club sucker greatly enlarged left | CSgeL | Number of greatly enlarged suckers on left tentacular club |
| Club sucker greatly enlarged right | CSgeR | Number of greatly enlarged suckers on right tentacular club |
| Club sucker diameter normal left | CSDnL | Diameter of normal suckers on left tentacular club |
| Club sucker diameter enlarged left | CSDeL | Diameter of enlarged suckers on left tentacular club |
| Club sucker diameter greatly enlarged left | CSDgeL | Diameter of greatly enlarged suckers on left tentacular club |
| Club sucker diameter normal right | CSDnR | Diameter of normal suckers on right tentacular club |
| Club sucker diameter enlarged right | CSDeR | Diameter of enlarged suckers on right tentacular club |
| Club sucker diameter greatly enlarged right | CSDgeR | Diameter of greatly enlarged suckers on right tentacular club |
| Tentacular club sucker left | CSL | Total number of suckers on left tentacular club |
| Tentacular club sucker right | CSR | Total Number of suckers on right tentacular club |
| Tentacular club sucker series left | CSSL | Number of transverse rows of suckers on left tentacular club |
| Tentacular club sucker series right | CSSR | Number of transverse rows of suckers on right tentacular club |
| Arm IV sucker | SA4 | Total number of suckers on right ventral arm |
| Weight | W | Total wet body weight (g) |

**Table 2** Cuttlebone morphometric characters of *Sepia pharaonis* Ehrenberg, 1831 used in this study (modified from Jereb et al. (2005) and Roper and Voss (1983))

| Character | Abbreviation | Definition |
|---|---|---|
| Cuttlebone length | CBL | Length of cuttlebone from anterior of cuttlebone to end of spine |
| Cuttlebone width | CBW | Greatest width of cuttlebone |
| Last loculus length | LLL | Length of smooth zone from tip of the last lamella to anterior cuttlebone on ventral surface |
| Striated zone length | STL | Length of striated zone from the first lamella to the last on ventral surface |
| Inner cone width | ICW | Greatest width of inner cone on ventral surface |
| Inner cone length | ICL | Greatest length of inner cone on ventral surface |
| Outer cone width | OCW | Greatest width of outer cone on ventral surface |
| Spine length | SL | Length of spine from tip to posterior apex of cuttlebone |

**Table 3** Digestive system morphometric characters of *Sepia pharaonis* Ehrenberg, 1831 used in this study (modified from Jereb et al. (2005), Roper and Voss (1983) and Dunning et al. (1998))

| Character | Abbreviation | Definition |
|---|---|---|
| Stomach length | SL | Length of stomach from posterior end of oesophagus to anterior point of intestine |
| Stomach width | SW | Greatest width of stomach |
| Oesophagus length | EL | Length of oesophagus from posterior buccal mass to anterior of stomach |
| Oesophagus width | EW | Greatest width of oesophagus |
| Right digestive gland length | DGLr | Length of right digestive gland from anterior tip to posterior tip of gland in parallel position to oesophagus |
| Left digestive gland length | DGLl | Length of left digestive gland from anterior tip to posterior tip of gland in parallel position to oesophagus |
| Right digestive gland width | DGWr | Greatest width of right digestive gland |
| Left digestive gland width | DGWl | Greatest width of left digestive gland |
| Caecum length | CCL | Length of ceacum from posterior of intestine to posterior tip |
| Caecum width | CCW | Greatest width of caecum |
| Ink sac length | ISL | Length of ink sac from anterior tip of sac to posterior rectum |

**Table 4** Reproductive system morphometric characters of *Sepia pharaonis* Ehrenberg, 1831 used in this study (modified from Jereb et al. (2005), Roper and Voss (1983) and Dunning et al. (1998))

| Character | Abbreviation | Definition |
|---|---|---|
| Ovary length | OL | Length of ovary from anterior to posterior tip |
| Ovary width | OW | Greatest width of ovary |
| Oviduct length | ODL | Length of oviduct from anterior to posterior tip |
| Oviduct width | ODW | Greatest width of oviduct |
| Nidamental gland length | NGL | Length of nidamantle gland from anterior to posterior tip |
| Nidamental gland width | NGW | Greatest width of nidamental gland |
| Spermatophoric sac length | SSL | Length of spermatophoric sac (Needham's sac) from anterior tip of sac to posterior tip of penis |
| Spermatophoric sac width | SSW | Greatest width of spermatophoric sac |
| Spermatophore count | SpC | Numbers of spermatophores in spermatophoric sac |
| Testis length | TeL | Length of testis from anterior to posterior tip of testis |
| Testis width | TeW | Greatest width of testis |

**Table 5** Hectocotylus morphological and morphometric characters of *Sepia pharaonis* Ehrenberg, 1831 used in this study (modified from Jereb et al. (2005) and Roper and Voss (1983))

| Character | Abbreviation | Definition |
|---|---|---|
| Hectocotylised arm length | HAL | Total length of left fourth arm from proximal sucker to distal tip of arm |
| Hectocotylus length | HcL | Length of modified part of hectocotylised arm |
| Modified sucker series | MSS | Number of transverse rows of modified (reduced size) suckers in medial region of arm |
| Proximal sucker series | PSS | Number of transverse rows of normal suckers on proximal part of arm |
| Distal sucker series | DSS | Number of transverse rows of normal suckers on distal part of arm |

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