The taxonomy of Vietnam's exploited seahorses (family Syngnathidae)

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Seahorses (*Hippocampus* spp) are heavily exploited in Vietnam, but conservation and management measures are currently limited by the ambiguous taxonomic definitions of the genus in the region. Seven species of seahorse are identified in this paper as inhabiting the coastal waters of Vietnam. We used morphometric and DNA sequence data (from the cytochrome *b* region of the mitochondrial genome) to delimit the species. Species descriptions are put forward and we provide illustrations and an identification key to the species. The species are provisionally assigned to *Hippocampus spinosissimus* Weber 1913, *H. comes* Cantor 1850, *H. trimaculatus* Leach 1814, *H. kuda* Bleeker 1852, *H. kelloggi* Jordan and Snyder 1902, *H. mohnikei* Bleeker 1854 and *H. histrix* Kaup 1856. The current level of confusion in seahorse nomenclature means that some of these distinct species in Vietnam (in particular *H. spinosissimus, H. kuda, H. kelloggi* and *H. mohnikei*) may have to be renamed once a comprehensive revision of the genus has been completed.

KEY WORDS:—Hippocampus – morphometrics – mtDNA – marine conservation.

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INTRODUCTION

A comprehensive revision of seahorse taxonomy is urgently needed (Vari, 1982; Paxton *et al.*, 1989; Gomon, 1997). The current chaos in seahorse taxonomy raises concern in light of the global exploitation of seahorses for traditional medicines, tonic foods, aphrodisiacs, aquarium fishes and curiosities (Vincent, 1996). As in other species, taxonomic definitions must be established before effective conservation measures can be applied to target organisms (Ihssen *et al.*, 1981). Our inability to determine the identities of seahorses restricts research on geographic ranges, population numbers and viability, hinders understanding of trade routes and consumer preferences for particular species, impedes conservation and management action (including trade monitoring and legislation), and the lack of standardized nomenclature hampers effective communication. Clear species identification will make it easier to modify fishing practices appropriately, design protective marine reserves, and assess captive breeding potential for seahorses.

Seahorses (genus *Hippocampus*) are members of the family Syngnathidae, which also includes pipefishes, pipehorses and seadragons. Seahorses are found worldwide in shallow coastal tropical and temperate seas from about 45°N to 45°S. McAllister (1990) lists 106 species names (excluding obvious mis-spellings), of which at least 50 claim to be species from the Indo-Pacific. Many of these are mis-identifications or have subsequently been inferred to be synonyms (McAllister, 1990; Eschmeyer, 1996). The confusion in seahorse taxonomy has four main sources: limited morphological variation among species, poor type descriptions, independent designation of the same name for different species, and seahorses' ability to camouflage themselves by changing colour and growing skin filaments to match their surroundings (A. Vincent, pers. obs.; H. Masonjones, pers. comm.).

Vietnam is among the larger exporters of seahorses globally, selling at least 5 tonnes of seahorses every year, predominantly to China (Vincent, 1996). Demand will continue to grow as China's economic boom promotes consumer spending on traditional medicines. The seahorse trade in Vietnam increased greatly during the 1980s with the consequence that seahorses are already reported to be declining in number and size (Vincent & Truong Si Ky, unpublished data). Seahorse characteristics of low fecundity, limited mobility, structured mating patterns and site fidelity, make them particularly vulnerable to to heavy fishing pressure. Four seahorse species (putative *H. histrix*, *H. kuda*, *H. kelloggi* and *H. trimaculatus*) are listed as Vulnerable in Vietnam's *Red Data Book* (Ministry of Science, 1992) while another species (*H. japonicus* (junior synonym of *H. mohnikei*) is described as insufficiently known. The four listed species were included because "there cannot be many in the sea", without any additional evidence; we cannot be confident of the identities of any of these species. The 1996 *IUCN Red List of Threatened Animals* (Baillie & Groombridge, 1996) includes most Indo-Pacific seahorse species. Some of the names are certainly synonyms but current policy requires that all be included until the taxonomy is resolved.

Combining morphometric and genetic evidence offers the most robust approach to determining taxonomy and phylogeny, especially for seahorses. Seahorses are conservative in morphology, lack certain key physical features (e.g. pelvic and caudal fins) often used in the morphometric analysis of other fish species, and variation in body proportions and meristics—used to determine species membership—often overlap among species. Such subtleties in species distinctions can lead to 'lumping' of multiple 'cryptic' species under the same name (Knowlton, 1993). Alternatively, morphologically distinct forms may be split into superfluous species when they actually represent polymorphisms of a single interbreeding population. Molecular markers, especially those that uncover fixed allelic differences at diagnostic loci, are proving increasingly valuable in identifying species (Bartlett & Davidson, 1991; Powers, 1991; Duellman & Hillis, 1987). In particular, non-recombining mito-chondrial DNA (mtDNA) has received strong support for its use as a marker in conservation issues (Avise, 1995; Moritz, 1994). Techniques for analysing mtDNA sequences for phylogeny reconstruction are well-established (Avise, 1986). The cytochrome *b* region of mtDNA is widely used for phylogenetic work (Meyer, 1994) and has been used to study relationships across a range of taxa including mammals (Stanley *et al.*, 1994), birds (Fehrer, 1996) and fishes (Sturmbauer *et al.*, 1994). For practical field-based conservation however, it is essential for species to be recognizable on the basis of unambiguous morphological criteria.

This paper provides the first detailed taxonomic revision of Vietnam's known seahorse species, based on morphometric and genetic analyses. We indicate the current inferred distribution of each species in Vietnam and supply illustrations and a provisional key to the species. Scientific names have been assigned after re-examination of original type specimens although we have tended to be conservative and retained old names pending the completion of a comprehensive revision of the genus.

MATERIAL AND METHODS

Seahorses were obtained from the entire length of Vietnam between June 1995 and June 1997 (Fig. 1). All seahorses had been caught for commercial sale on the open market as medicines and curios, with none caught specifically for our research. Most came from offshore trawlers, but seahorses of morphotype D were hand-collected, usually by divers in estuaries. Seahorses were selected at random from among those landed by fishers. We sorted the seahorses by eye into seven apparent morphotypes, assigning to each morphotype a letter and to each individual seahorse a unique number. The genetic analysis is based on a subset of those morphotyped; we tried to include extremes of form and geographical range.

Morphometrics

We measured 100 dried seahorses: Morphotype A, $n = 25$; B, $n = 8$; C, $n = 20$; D, $n = 15$; E, $n = 14$; F, $n = 4$; G, $n = 9$ (Table 1). Outliers (5 *H. abdominalis* from Australia and New Zealand) were also included to test the discriminatory power of the multivariate analysis (as per Iliadou *et al.*, 1996). Morphometric measurements (Fig. 2) were taken using dial callipers to the nearest 0.1 mm, on the right side of the
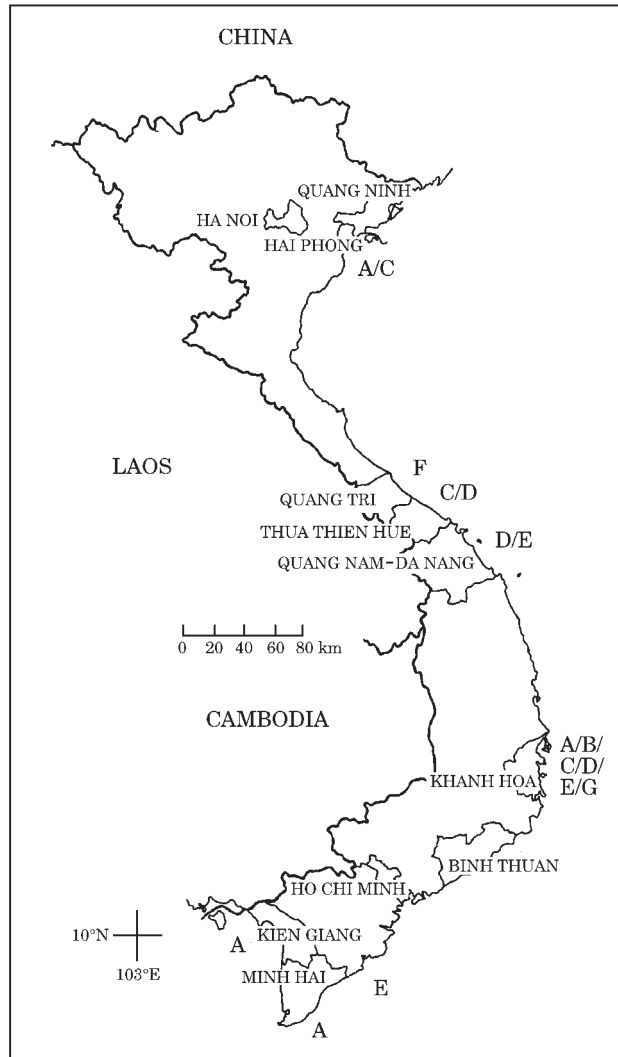
Figure 1. Map of Vietnam showing the coastal areas in which the seahorses in this study were caught, and other provinces mentioned in the text. Letters indicate the seahorse morphotypes.

seahorse, and repeated to ensure accuracy (to within 0.1 mm or 2.5% of the measured value) and the mean taken. Fin rays and tail rings were counted under a binocular microscope. Juveniles and specimens with missing values were excluded from the Principle Components Analysis (PCA), but were included in formulating the species descriptions. Those with missing values were included, wherever possible, in Table 2 of the results. We provide full details of our protocol in the hope of standardizing measurement of these unusual fishes (Appendix 1).

Morphometric data were analysed using sheared PCA as detailed in Humphries *et al.* (1981) and Bookstein *et al.* (1985). This technique, which quantifies shape differences independent of size, has previously been used to distinguish fish species (Strauss, 1985; Stauffer, 1991; Bowers & Stauffer, 1993).

TABLE 1. Seahorse specimens used in our study. #Only a single Morphotype B male was available. Juveniles and those with missing values were excluded from the multivariate analyses although included in compiling the species descriptions (a † symbol represents each specimen). +Both Morphotype E juveniles were included as females in the PCA

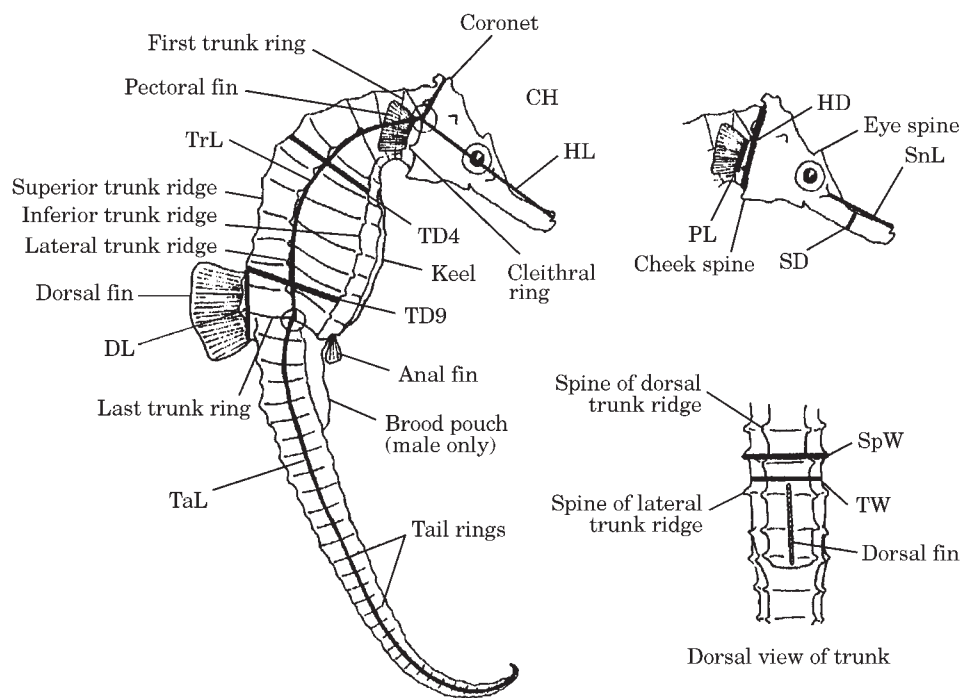| Morphotype	Putative species	Morphometrics			Genetics	
		male	female	juvenile?	male	female
A	H. spinosissimus	12†	11	2		2
B	H. comes	1#	7		1	2
C	H. trimaculatus	10	10		1	4
D	H. kuda	7	7	1	1	2
E	H. kelloggi	6	6	2+	2	3
F	H. mohnikei	2††	2		1	
G	H. histrix	4	4	1	3	1
	H. abdominalis	1	4			
TOTALS		43	51	6	9	14

Figure 2. Morphometric measurements used in this study (for full explanation see Appendix 1): TrL (trunk length); TaL (tail length); CH (coronet height); HL (head length); SnL (snout length); SD (snout depth); HD (head depth); TD4 (trunk depth between 4th and 5th rings); TD9 (trunk depth between 9th and 10th rings); TW (trunk width between 9th and 10th rings); SpW (trunk width at the 9th ring including lateral spines); PL (pectoral fin base length); DL (dorsal fin base length). SL (standard length) is defined as HL + TrL + TaL. Meristic counts: TrR (trunk rings); TaR (tail rings); PF (pectoral fin rays); DF (dorsal fin rays). Indices: SI (spininess); CI (coronet height); KI (keel depth); CSI (chin shape) (see Figs A1 and A2 in Appendix 1). Seahorse redrawn from Kim & Lee (1995).

Morphometric measurements were log transformed to preserve allometries, stand-ardize variances and produce a scale-invariant covariance matrix before analysis (Jolicoeur, 1963). All multivariate analyses were carried out using the R-package (Legendre & Vaudor, 1991).

Morphometric data and meristics/indices were analysed separately (Humphries *et al.*, 1981). To ensure a comprehensive analysis of the data and for more powerful discrimination between groups, sheared principal components representing both (a) morphometric measures and (b) the size-independent shape factor were scattered against the principal components of the correlation matrix of discontinuous characters (meristics and indices) (Humphries *et al.*, 1981).

Cluster analyses were carried out using the SIMIL and CLUSTER programmes of the R-package (Legendre & Vaudor, 1991). Gower's similarity matrices were created from logged morphometric data, logged meristic data and indices (coded as qualitative multiclass descriptors) (Gower, 1985). These matrices were then subjected to the agglomerative clustering algorithm UPGMA (unweighted pair group matrix algorithm) of Lance & Williams (1967).

Using both PCA and Cluster analytical methods gave us two independent tests for the robustness of our morphometric groupings: PCA or factor analyses do not presume multiple groups and thus allow for their discovery (Humphries *et al.*, 1981), whereas cluster analyses force individuals into groups.

Examination of type material

The original type specimens of all plausible Vietnamese species had to be re-examined because many seahorse type descriptions are inadequate, citing only (changeable) colour and skin filaments as diagnostic features. We examined type material from the Natural History Museum, London (BMNH), Museum National d'Histoire Naturelle, Paris (MNHN), Nationaal Natuurhistorisch Museum, Leiden (RMNH), California Academy of Sciences (CAS) and the Zoologisch Museum, Amsterdam (ZMA). Details are given with the species descriptions in Appendix 3. Morphometric and meristic data from type specimens were used to assess putative species membership of our morphotypes.

Genetics

We compared 354 base pairs of sequence from the 5′ end of the mitochondrial cytochrome *b* gene of 23 individual dried seahorses: Morphotype A, $n=2$; B, $n=3$; C, $n=5$; D, $n=3$; E, $n=5$; F, $n=1$; G, $n=4$. Unfortunately, due to technical difficulties genetic data were only obtained from a single Morphotype F specimen and two Morph A specimens.

DNA was extracted from 50 mg of tail muscle tissue (high mitochondrial content) using a standard proteinase K/phenol method (Sambrook *et al.*, 1989). The entire cytochrome *b* region (1141 base pairs) was amplified by polymerase chain reaction (PCR) (Mullis & Faloona, 1987) using the universal primers L14724 and H15915 (Irwin *et al.*, 1991) and the following cycle parameters: 94°C 45 sec, 50°C 45 sec, 72°C 2.5 min (30 cycles), followed by 72°C 10 min, in a Perkin Elmer Cetus Model 9600 thermal cycler. Individual reactions were performed in a total volume of 25 µl

containing 10 mM TrisHCL (pH 8.8) 50 mM Kcl$_2$, 1.5 mM MgCl$_2$, 1% non-ionic detergent, 100 μM each of dGTP, dATP, dTTP and dCTP, 1U *Pfu* DNA polymerase (Stratagene) and 12.5 pmol of each primer.

Products of successful amplifications were cloned using the pCR-Script™ Amp SK(+) Cloning Kit (Stratagene). Plasmid DNA was purified using a Stratagene ClearCut™ Miniprep Kit, sequenced using an ABI PRISM™ Dye Terminator Cycle Sequencing Reaction Kit, and T3 or T7 primers (Stratagene), and run on an ABI377 system.

Sequences were aligned and edited using Sequencher 3.0 (Gene Codes Corporation, Inc.) and MacClade 3.03 (Maddison & Maddison, 1993). Parsimony analysis was conducted using PAUP 3.1.1 (Swofford, 1990), and support for the monophyly of each morphotype group was assessed using the bootstrap procedure (Felsenstein, 1985). A Neighbour-joining tree was constructed using PHYLIP (Felsenstein, 1991).

RESULTS

Morphometrics: sheared PCA

Variation in overall body shape, relative snout length, coronet height, number of tail rings, 'chin shape' and degree of development of body and tail spines was sufficient to separate the specimens by eye into morphotypes (Table 1). Basic morphological statistics for each morphotype are provided in Table 2, using abbreviations given in Figure 2.

When the sheared principal components (representing the morphometric measures and size-independent shape factor) were scattered against the principal components of the correlation matrix of the discrete (meristic and index) characters, individuals of morphotype C, F, G and *H. abdominalis* (females) clustered out distinctly and discretely in ordination space (Fig. 3; Tables 3, 4). The meristic data for the male *H. abdominalis* differed from that of the females and thus pulled the group apart.

Morphotypes A, B, D, and to a lesser extent E, overlap. PCA is notoriously sensitive to outliers, causing relatively similar species to clump together even though they may in fact be distinct (Dunn & Everitt, 1982; Tabachnik & Fidell, 1989). We therefore removed individuals belonging to the outlying groups (C, F, G and *H. abdominalis*) from the dataset. New sheared PCA and meristic PCA of the remaining data allowed increased resolution of the central overlapping region (Fig. 4; Tables 5, 6). The resulting groupings show no overlap between morphotypes. However, the inter-versus intra-group distances are too small to conclude confidently (they are distinct from each other) from morphometric data alone but these groupings are substantiated by genetic analysis.

Sexual dimorphism

Male and female data were also analysed separately, following the above procedures, and the same morphotype groupings appeared indicating that variation due to sexual dimorphism is much less than that among morphotypes. The effect of sex was evident as shape rather than size variation (as noted in Vincent, 1990)

TABLE 2. Basic morphometric statistics for Vietnamese seahorse species (males and females combined)

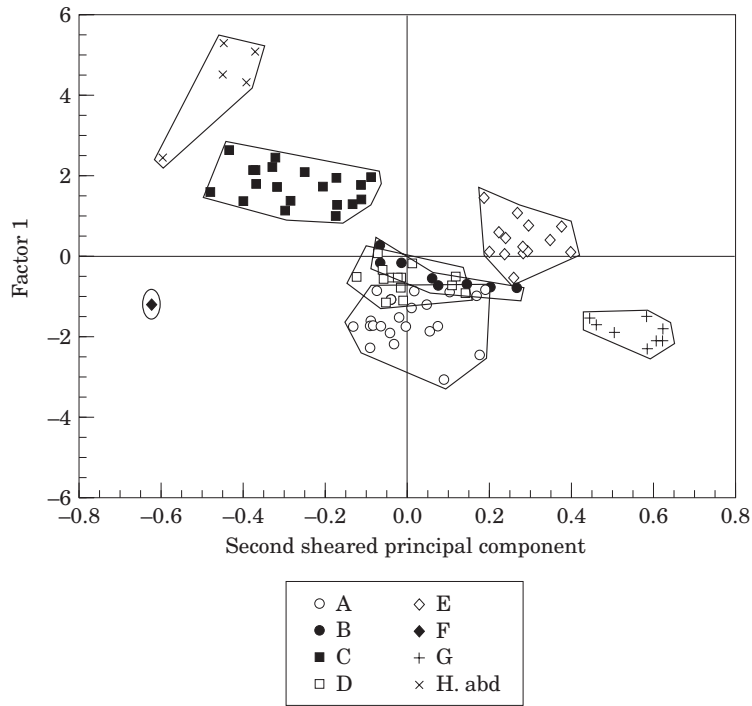| Morphotype	A	B	C	D	E	F	G
Putative species	*H. spinosissimus*	*H. comes*	*H. trimaculatus*	*H. kuda*	*H. kelloggi*	*H. mohnikei*	*H. histrix*
n	23	8	20	14	12	4	8
	(22 for TW)					(3 for PF, 2 for DF, StL, TrL, TaL, HL, SnL, CH, SnD)	
MORPHOMETRICS							
SL (mean ± SD)	135.0 ± 19.4	157.8 ± 10.1	130.0 ± 16.8	148.0 ± 13.5	198.5 ± 43.3	55.3 ± 2.1	110.5 ± 16.5
TrL (%StL)	25.8 ± 1.2	27.1 ± 1.4	24.7 ± 1.1	26.1 ± 1.0	27.5 ± 1.2	25.4 ± 0.9	29.0 ± 1.4
TaL (%StL)	54.6 ± 2.1	52.4 ± 2.3	56.8 ± 1.8	54.7 ± 1.3	52.7 ± 1.7	57.0 ± 0.4	47.0 ± 1.9
HL (%StL)	19.7 ± 1.4	20.5 ± 1.2	18.4 ± 1.1	19.2 ± 0.9	19.7 ± 0.8	17.6 ± 1.2	24.0 ± 0.9
SnL (%HL)	44.7 ± 1.5	47.1 ± 2.2	44.5 ± 2.2	43.1 ± 1.9	47.6 ± 1.1	27.1 ± 1.8	55.7 ± 1.6
CH (%HD)	63.8 ± 4.4	52.2 ± 2.0	54.6 ± 2.8	60.3 ± 2.9	63.7 ± 2.7	60.8 ± 4.1	72.1 ± 2.1
HD (%HL)	46.6 ± 3.2	40.4 ± 1.6	45.3 ± 3.2	49.4 ± 1.9	45.5 ± 1.7	58.7 ± 2.5	35.6 ± 1.8
SnD (%SnL)	24.2 ± 2.6	20.5 ± 2.3	20.4 ± 2.8	26.4 ± 2.2	22.5 ± 1.7	61.5 ± 6.5	11.9 ± 1.1
TD4 (%TD9)	72.7 ± 6.5	66.6 ± 3.1	69.2 ± 7.0	67.5 ± 6.5	82.3 ± 4.7	84.6 ± 10.8	85.9 ± 9.3
TW (%SpW)	69.1 ± 2.9	82.3 ± 4.5	86.3 ± 2.9	84.1 ± 5.3	76.8 ± 4.0	75.2 ± 2.8	49.5 ± 2.6
DL (%TrL)	27.5 ± 3.3	25.2 ± 2.4	34.5 ± 2.4	26.7 ± 2.1	25.0 ± 1.5	30.6 ± 5.6	18.0 ± 1.7
PL (%HD)	36.8 ± 2.1	36.8 ± 1.5	37.2 ± 2.1	32.3 ± 2.1	37.5 ± 4.6	27.1 ± 6.2	36.2 ± 1.9
MERISTICS							
No. Tr rings (TaR) (mode)	11	11	11	11	11	11	11
No. Ta rings (TaR)	37	35	41	36	40	36	35
Pectoral fin rays (PF)	17	17	18	16	18	13	18
Dorsal fin rays (DF)	18	18	20	17	18	15	17
INDICES							
Spininess (SI) (mode)	2	2	1	1	1	2	3
Coronet (CI)	3	2	1	2	3	2	2
Keel (KI)	1	1	3	2	1	2	2
Chin shape (CSI)	1	5	3	2	4	5	1

Figure 3. Plot of first factor score (meristic data) and second sheared principle component (morphometric data) for Morphotypes A–G and *H. abdominalis*.

TABLE 3. Morphometric variable loadings for the principle component analyses of putative Vietnamese seahorse species. (λ represents the eigenvalue or % total variation accounted for by each principal component). † gives the regression parameter values used to calculate the sheared PCII from Principle Components I and II where sheared PCII $=$ PCI $(-\alpha\beta_1)$ $+$ PCII $(1- (-\alpha\beta_2)$

Character	Principal component I ($\lambda=.79$ or 81.3%)	Principal component II ($\lambda=.08$ or 8.6%)	Sheared PCII
TrL	0.29	0.13	0.14
TaL	0.30	-0.23	-0.22
HL	0.26	0.28	0.29
SnL	0.29	0.61	0.62
CH	0.24	0.27	0.28
HD	0.27	0.03	0.04
SnD	0.30	0.02	0.03
TD4	0.23	-0.01	*
TD9	0.29	-0.29	-0.28
TW	0.31	-0.13	-0.11
DL	0.35	-0.54	-0.53
PL	0.31	0.08	0.09

†$\alpha = -.043$, $\beta_1 = 1.001$, $\beta_2 = -.045$
Loadings $< \pm 0.01$ are not shown (*)

(two-way ANOVA on the object loadings of the second principle component [mainly shape information] was significant for sex as a main effect: $F=22.31$, df$=13$, $P=0.00$, whereas a similar test on the first priciple component [mainly size information] was not significant: $F=1.34$, df$=13$, $P=0.25$). Trunk length, as a proportion of

TABLE 4. Meristic variable loadings for the principle component analyses of putative Vietnamese seahorse species. (λ represents the eigenvalue or % total variation accounted for by each principal component)

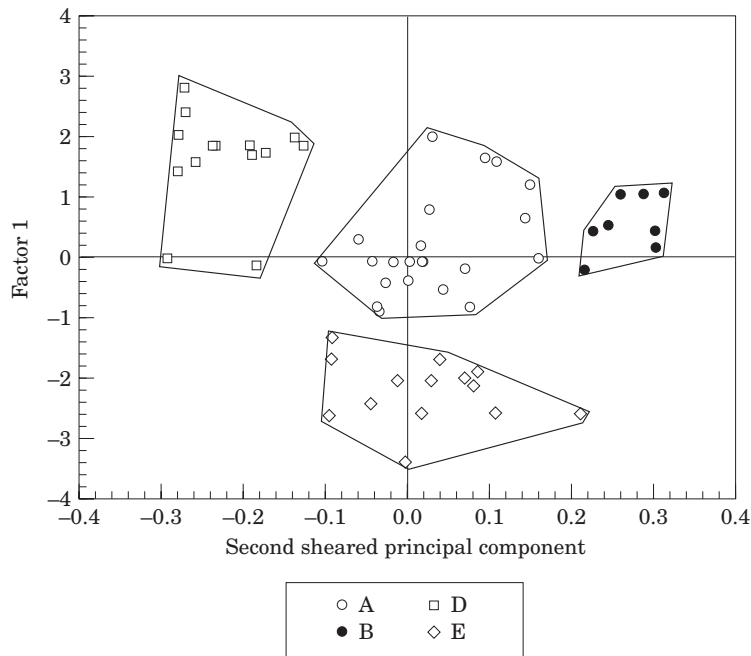| Character	Principal component I (λ=2.91 or 41.5%)	Principal component II (λ=1.18 or 16.9%)
TaR	0.51	0.02
PF	0.05	−0.47
DF	0.50	0.03
SI	−0.39	−0.30
CI	−0.36	0.39
KI	0.30	−0.51
CSI	0.34	0.52

Figure 4. Plot of first factor score (meristic data) and second sheared principal component (morphometric data) for Morphotypes A, B, D and E after removing clear groups (Morphotypes G, C, F and *H. abdominalis*).

standard length, was greater for females than males in all species for which sufficient data was available for comparative analysis (Morphotypes A, $n=11$ (females) and 12 (males); C, $n=10$, 10; D, $n=7$, 7; E, $n=6$, 6; and G, $n=4$, 4. Wilcoxon test $P=0.002$, 0.001, 0.019, 0.047, 0.015 respectively). Males also tended to be deeper-bodied in Morphotypes A, C and D (Wilcoxon test, $P=0.001$, 0.001, 0.006 respectively) and have longer dorsal fin bases in relation to trunk length than females (Wilcoxon test, $P=0.002$, 0.006, 0.000). Spine length was dimorphic in Morphotypes C and G (females had better developed spines in Morphotype C, whereas males did in Morphotype G. Wilcoxon test $P=0.014$ for both species). Standard length only differed significantly between the sexes in Morphotype A—males being the larger sex (Wilcoxon test, $P=0.003$).

Table 5. Morphometric variable loadings for the principle component analyses of putative Vietnamese seahorse species, Morphotypes A, B, D and E (after removal of clear groups). (λ and † as for Table 3)

Character	Principal component I ($\lambda = .41$ or 82.4%)	Principal component II ($\lambda = .03$ or 5.5%)	Sheared PCII
TrL	0.31	0.12	0.13
TaL	0.29	−0.09	−0.09
HL	0.30	0.22	0.22
SnL	0.33	0.33	0.33
CH	0.26	−0.43	−0.43
HD	0.28	−0.24	−0.23
SnD	0.31	−0.12	−0.12
TD4	0.25	−0.21	−0.20
TD9	0.24	−0.29	−0.28
TW	0.23	0.66	0.67
DL	0.34	−0.02	−0.02
PL	0.31	0.03	0.03

†$\alpha = -0.02$, $\beta_1 = 1.01$, $\beta_2 = -0.02$

Table 6. Meristic variable loadings for the principle component analyses of putative Vietnamese seahorse species, Morphotypes A, B, D and E (after removal of clear groups)

Character	Principal component I ($\lambda = 2.39$ or 34.2%)	Principal component II ($\lambda = 1.72$ or 24.5%)
TaR	−0.53	−0.18
PF	−0.57	0.04
DF	−0.39	−0.08
SI	0.02	0.68
CI	−0.38	0.31
KI	0.09	−0.61
CSI	−0.31	−0.20

Cluster analysis

Nearly 98% ($n = 90/92$) of specimens clustered into the groups we had predicted in our morphotype designations. When the sexes were analysed separately, 98% of females ($n = 51/52$) and 100% of males ($n = 40$) clustered into the morphotype groups.

Genetics

Of the 354 base pairs analysed, 108 sites were variable among taxa (96 sites parsimony-informative). Fourteen haplotypes were observed among the 23 specimens. No insertions or deletions were detected. The observed patterns of nucleotide composition and variation were typical of those found in cytochrome *b* studies of other fish species (Lydeard & Roe, 1997) with substitutions occurring predominantly at the third position of codons, a high transition/transversion ratio (5:1) at variable sites, and a low guanine content. Maximum sequence divergence among all specimens was 19.4% (corrected Kimura 2-parameter distance measure; Kimura, 1980).
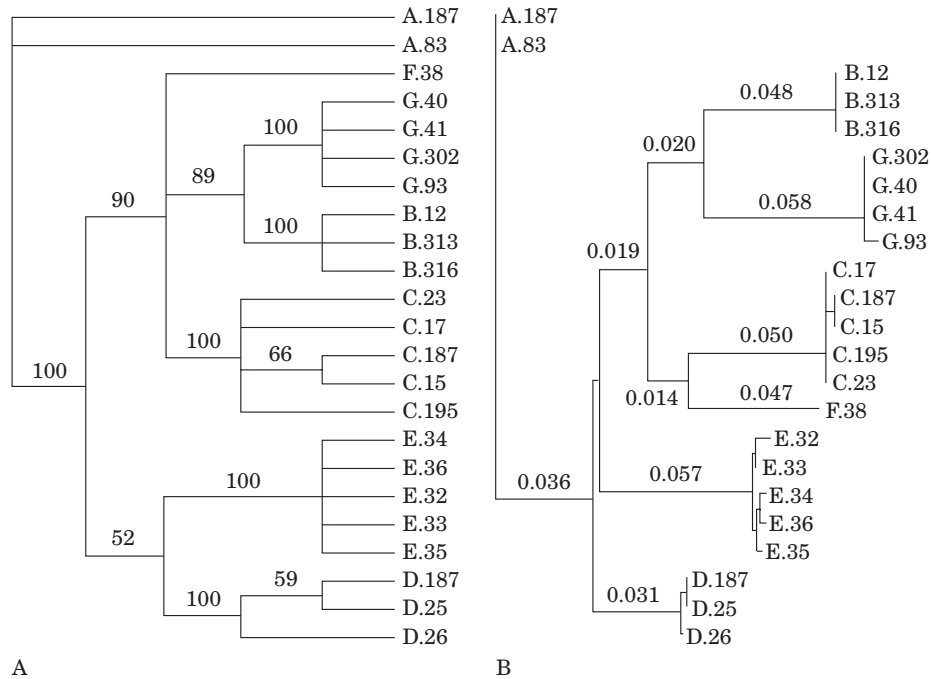
Figure 5. A, PAUP Bootstrap consensus tree (1000 replications). Bootstrap support is indicated above branches; B, PHYLIP Neighbour-joining tree based on the Kimura (1980) two-parameter distance (K2P). Numbers above branches are K2P distances, with branch length proportional to distance (branch lengths of less than 0.01 are not marked; zero length branches are collapsed). K2P distances within a single morphotype group did not exceed 0.005. Note that both trees are arbitrarily rooted with Morphotype A.

Divergence between morphotypes was 7.2–19.4%, while variation within any single morphotype was less than 1.0%. Maximum parsimony analysis (heuristic search) produced four trees, and bootstrap resampling (1000 replications) was used to produce a consensus tree (Fig. 5). The grouping of the 23 specimens into seven distinct morphotypes received very high bootstrap support. A Neighbour-joining tree was created to illustrate the genetic distances among taxa (Fig. 5).

Representative sequences for each of the morphotypes have been deposited in GenBank with accession numbers AF033004 to AF033010.

<div align="center">DISCUSSION</div>

Our work shows that Vietnam has at least seven species of seahorses (Appendix 3), which is more than has been claimed for most nations (see Vincent, 1996) even within the Indo-Pacific. Previous papers have cited only two species: *H. kuda* and *H. histrix* (spelt 'shitrix') nationally (Kuronuma, 1961) or five seahorses species in the Gulf of Tonkin: *H. histrix* (Morphotype A), *H. trimaculatus*, *H. kuda*, *H. kelloggi* and *H. japonicus* (junior synonym of *H. mohnikei*) (Nguyen Khac Huong, 1993). Such high species richness as we have shown might be surprising given Vietnam's relatively straight coastline, narrow continental shelf for much of its length, and dearth of

islands and archipelagos. This apparent over-representation may, however, reflect lack of research elsewhere rather than a biological reality.

Morphological variation was sufficient to distinguish the seven Vietnamese seahorse morphotypes, although inter-group distances were sometimes small. We have highlighted characters which permit species identification by eye and this should facilitate practical application of our results in the field (allowing determination of species ranges) and in markets (permitting estimates of trade volumes per species, and understanding of trade routes). We found concordance between morphological and genetic analysis. The magnitude of genetic distances within and among the morphotypes supported our designations of the morphotypes as separate species.

Examination of type specimens allows us to propose species names which differ from those currently in use in Vietnam (Si Ky Truong, pers. comm.): (1) *H. histrix* is the species name for Morphotype G, not for Morphotype A as had been commonly assumed (2) Morphotype A is instead putative *H. spinosissimus* and (3) Morphotype F should be called putative *H. mohnikei* and not *H. spinosissimus* as now. These changes should be noted as quickly as possible to reduce further confusion. Misidentification as, for example, in the recent listing of seahorse species in the Vietnamese *Red Data Book* (Ministry of Science, 1992), where the pictures illustrating *H. trimaculatus* and *H. kelloggi* both appear to represent Morphotype C, may have serious consequences.

Two species of seahorse appear to be particularly common and widely-distributed in Vietnam: Morphotype A (*H. spinosissimus*) and Morphotype C (*H. trimaculatus*). We do not yet have detailed information about species ranges, but species composition certainly varies geographically. *H. trimaculatus* comprises 86% of all seahorses caught in Quangninh province, while *H. spinosissimus* and *H. trimaculatus* comprise 56% and 44% of the catch respectively in Binhthuan province (Truong Si Ky, pers. comm.) It is clear that Morphotype A is found nationally but data are too sparse for accurate mapping, especially as seahorses may be caught some distance from where they are brought ashore.

We are aware of the potential fallacy that: "two groups need not necessarily be the same simply because an analysis based on a particular data set has not shown them to be different" (Strauss & Bookstein, 1982). Indeed, considerable morphological variability in Morphotype A (putative *H. spinosissimus*) suggests that it could still encompass a complex of more than one species. For example, two of our specimens, both dark red, had very fine spines and tiny coronets, although they were indistinguishable from the other Morphotype A specimens in terms of meristics and morphological measurements. It is entirely possible that such variability is due to developmental changes or environmental factors but we would need more material before we could confidently conclude that Morphotype A is a single species.

Morphotype B (putative *H. comes*) has never before been reported from Vietnam. We named it B because of it looked similar to our previously morphotyped Morphotype B from the Philippines. Morphological variation between these two groups in slight but consistent—cluster analysis joined the groups but PCA separated them, although the inter-group distance was small (unpublished data)—and may reflect a geographical cline if it does not warrant specific separation.

There appears to be consensus regarding the designation of Morphotype C as *H. trimaculatus*. Although not all specimens have the characteristic '3-spots' on their dorsolateral trunk surface (see Appendix 3) the DNA sequence data were identical for those with and without spots ($n = 2, 2$). Specimens which resemble Morphotype C have occasionally been labelled as *H. kelloggi* (Ministry of Science, 1992) but this is incorrect.

Morphotype D can be easily distinguished by eye from the other Vietnamese species, and the three specimens genetically analysed clustered tightly together. We have retained the name *H. kuda* for Morphotype D although there may still be some question about this. The morphometrics do overlap with a number of the specimens in the RMNH labelled as "believed to include the type specimen of *H. kuda*". The original description (Bleeker, 1854) refers to specimens from Singapore, so our Vietnamese morphotype may be a geographical variant. Globally, *H. kuda* poses a serious problem because the name is used very loosely for a range of morphotypes. In the literature it is quoted as being a widespread and variable species found "throughout the Indo-Pacific, from South and East Africa to Hawaii." (Bellomy, 1982). At least 33 species names have been synonymized with *H. kuda* (Weber & Beaufort, 1922), perhaps prematurely. In any case we are unwilling to rename this Vietnamese species until the complete 'kuda' complex has been reanalysed.

Morphotype E is tentatively assigned to *H. kelloggi*. We disagree with Lee's (1983) synonymization of *H. kelloggi* with *H. kuda* and the Vietnamese *Red Data Book* apparent synonymization of *H. kelloggi* with *H. trimaculatus* (Ministry of Science, 1992). Certainly these are three distinct species in Vietnam based on our morphometric and genetic data.

Only four Morphotype F specimens, in poor condition, were available for our study. Due to technical difficulties genetic data were only obtained for a single Morphotype F specimen. Morphometrically, they appear closest to the type specimens of *H. mohnikei* Bleeker 1854 and *H. japonicus* Kaup 1856 from Japan. Re-examination of the type specimens of both species suggests that they represent the same species (i.e. are synonyms), and therefore, according to the International Code of Zoological Nomenclature, the earlier name (i.e. *H. mohnikei*) should be retained.

Morphotype G is undoubtedly *H. histrix* Kaup 1856, despite this name most commonly being attributed to Morphotype A in Vietnam and other morphotypes elsewhere.

Dried and spirit-preserved seahorses were used in the morphometric analyses in this study. A comparative study has shown that dried and spirit-preserved specimens of the same morphotype cluster together in the analyses used here (J. Pritchard, unpublished data). Given this information, as seahorses have a rigid external skeleton of bony plates which permit little change in shape and size, the morphometric results can be justifiably extrapolated to fresh specimens and hence be of use in identifying live seahorses. The main difference noted in live specimens might be the presence of skin appendages and encrusting algae which may mask some of the body features, such as markings or spines. These appendages usually fall off soon after death (pers. obs.).

A detailed revision of the *Hippocampus* species in the Indo-Pacific is urgently needed. This paper should be viewed as a first stage in this process. We have demonstrated the validity of our methods, determined the current species divisions of seahorses in Vietnam, and clarified the nomenclature of four species whose names were previously confused (*H. spinosissimus, H. kelloggi, H. mohnikei, H. histrix*). Such standardization is essential for conservation purposes; enabling effective communication among fishers, traders, scientists, fisheries officers and customs officials. Taxonomic confusion complicates assessment of species' conservation status, forcing unnecessary listing of synonyms in IUCN Red Lists of Threatened Species and greatly increasing the possibility of omitting species at risk.

The need for clear species definitions is growing rapidly: the European Union

announced wildlife trade regulations in 1997 that require all seahorse imports be formally reported, and Australia now requires export permits for all seahorses (as of 1 January 1998) under a revision of the Wildlife Protection Act (1981). In addition to helping clarify seahorse taxonomy, our paper provides the basis on which to map geographic ranges and track trade volumes of individual species in Vietnam. Such research will be vital to assess fisheries impact on the seahorses of Vietnam, to clarify their conservation status, and to propose management options. Moreover, the taxonomy developed in this paper can be useful throughout the Indo-Pacific region, as many of these species are also found and exploited in other nations' waters.

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APPENDIX 1: SEAHORSE MEASUREMENT PROTOCOL

Seahorses are morphologically very unusual fish that defy traditional ichthyological measurement and meristic evaluation: their head is bent at right angles to their body, their trunk inherently curved, their body armour-plated and their tail prehensile. They also lack many of the key physical features (such as pelvic, caudal and second dorsal fins) often used in the morphometric analysis of other fish species. We give full details of our measurement protocol in order to standardize the measurement of seahorses (see Fig. 2 in Methods section and Figs A1 and A2 below). Measurements and counts should be taken on the right hand side where possible.

1. Standard length (SL) is defined as the sum of head length + trunk length + tail length (see below), such that it is comparable with this measurement in other fish (i.e. snout tip to hypural joint).

2. Trunk length (TrL) is defined as the distance from the point on the cleithral ring where it is joined by a diagonal ridge from the first trunk ring (circled in Fig. 2), to the mid-point of the lateral ridge of the last trunk ring (that immediately above the anal fin). To compensate for the curvature of the body of dried specimens, a piece of fine wire (<0.1 mm diameter) was used to mould around the curvature of the body. This was then straightened, laid flat and measured with callipers. Live or well-preserved specimens can usually be straightened enough to make the use of wire unnecessary.

3. Tail length (TaL): from the mid-point of the lateral ridge of the last trunk ring, to the tail tip. In

Snout

Eye
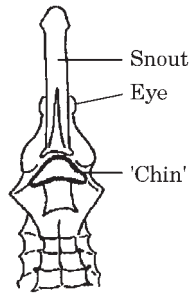
'Chin'

Ventral view of head
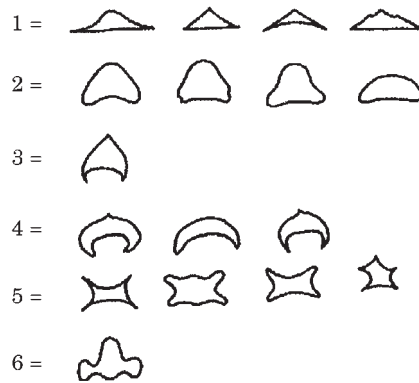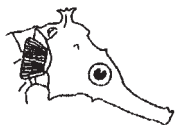
1 =

2 =

3 =

4 =

5 =

6 =

Figure A2. Chin shape index.

1 = coronet equal to
line of arc of neck

2 = coronet slightly raised
above line of arc of neck

3 = coronet significantly raised
above line of arc of neck

4 = coronet very tall

Figure A1. Coronet height index.

dried specimens again it was necessary to use a piece of wire as the tails could not be straightened.

4. Coronet height (CH): from the median groove of the coronet to the point on the cleithral ring where it is joined by a diagonal ridge from the first trunk ring.

5. Head length (HL): from the tip of the snout (upper part) to the point on the cleithral ring where it is joined by a diagonal ridge from the first trunk ring.

6. Snout length (SnL): from the tip of the snout (upper part) to the anterior side of the tubercle in front of the orbit.

7. Snout depth (SD): vertical measurement at its narrowest point.

8. Head depth (HD): from the lowest point of the depression immediately behind the coronet to immediately behind the cheek spine at the bottom of the cleithral ring.

9. Trunk depth between 4th and 5th trunk rings (TD4): narrowest distance between the superior and inferior trunk ridges between the 4th and 5th trunk rings.

10. Trunk depth between 9th and 10th trunk rings (TD9): narrowest distance between the superior and inferior trunk ridges between the 9th and 10th trunk rings. The ratio between these two trunk depth measurements allowed quantification of body shape.

11. Trunk width between 9th and 10th trunk rings (TW): narrowest distance between the left and right lateral trunk ridges between the 9th and 10th trunk rings. (This is most easily done from the back of the seahorse).

12. Distance between the 9th trunk ring lateral ridge spine tips (SpW): as for TW but with callipers closed onto the tips of the 9th trunk ring lateral spines.

13. Length of pectoral fin base (PL): distance between the dorsal and ventral angles of insertion of the fin rays.

14. Length of dorsal fin base (DL): as for PL.

15. Number of trunk rings (TrR).

16. Number of tail rings (TaR).

17. Number of pectoral fin rays (PF).

18. Number of dorsal fin rays (DF).

19. Ventral keel index (KI): the keel being the extra region of skin perpendicular to the ventral plates of the skeleton. This appears to be a sexually dimorphic trait, more common in males [1=no ventral keel, 2=ventral keel <1 mm deep, 3=ventral keel 1–3 mm deep, 4=ventral keel >3 mm deep].

20. Coronet height index (CI) (Fig. A1).

21. Spininess index (SI): for the first few spines on dorsal surface of tail (ignore larger ones where spines run in a series) [1=sum of spine lengths on left + right side ≤ 1/4 dorsal width of that tail segment, 2=length of spines >1/4 but ≤3/4 dorsal width, 3=length of spines >3/4 dorsal width].

22. Chin shape index (CSI) (Fig. A2).

APPENDIX 2: PROVISIONAL KEY TO VIETNAMESE SEAHORSE SPECIES

Species names marked † should be regarded as provisional until a detailed revision of the entire genus is published.

1. Thirty-nine or more tail rings 2
   Thirty-eight or fewer tail rings 3

2. Low coronet, angled backwards with 4 or 5 tiny points, spines above eye
      and below opercular ring sharply recurved, often with 3 dark spots
      on dorsal surface of 1st, 4th and 7th trunk ring (not always present)
                                                           .... *H. trimaculatus*

   Obviously raised coronet, prominent but rounded spine above eye and below
      cleithral ring, narrow trunk with thick rings   .... *H. kelloggi*†

3. Junctions of body ridges with pronounced spines 4
   Junctions of body ridges with knob-like tubercles or barely raised
                                                          5

4. Dorsal spines longer than width of body, very sharp and often black-tipped,
      narrow snout more than 0.5 × head length, black-edged keel,
      especially in males   .... *H. histrix*
   Dorsal spines variably developed, snout less than, or about 0.5 × head length,
      no keel, may have double spines beneath opercular ring .... *H. spinosissimus*†

5. Snout very short, much less than 0.5 × head length, adult body size
      less than 6 cm   .... *H. mohnikei*†
   Snout about 0.5 × head length, adult body size 12–16 cm 6

6. Junctions of body ridges with knob-like tubercles, striped tail (colours may be
      very muted), low coronet with 5 distinct rounded knob-like points .... *H. comes*
   Junctions of body ridges without prominent tubercles, rounded coronet,
      cheek spines rounded and not very prominent   .... *H. kuda*†

APPENDIX 3: SPECIES DESCRIPTIONS

   The number of dorsal (D) and pectoral (P) fin rays, and number of trunk + tail rings are given as ranges with the modal value in brackets. Full synonymies will be given when a comprehensive revision is published.

Morphotype A—Hippocampus spinosissimus Weber 1913 (*Fig. A3a*)

Materials. Eleven females, 12 males, 2 probable juveniles (not included in analyses).

Type specimens examined. H. spinosissimus Weber, 1913: ZMA 104655 (2) (from Sapeh Strait, Indonesia). *H. erinaceus* Günther 1870: BMNH 1855.9.19.1385 (Haslar Collection, no locality given).

D 17–19(18); P 16–18(17); Rings 11+36–37(37)

Description. Snout shorter than rest of head. Coronet high with 4–5 spines. Spines on junctions of body ridges well developed; those on 1st, 4th, 7th and 11th trunk rings and usually on 4th, 8th and 11th tail rings slightly more prominent. Younger specimens tend to have sharper spines. May have double cheek spines bordering throat at base of cleithral ring. Large males have very prominent spines bordering pouch.

Colour. Light yellow, brown, very dark red.

Standard length. 113–187 mm (juveniles 89 and 108 mm).

Habitat. Mud and sand, coral reef.

Inferred distribution. Quang Ninh, Hai Phong, Da Nang and from Khanh Hoa to Kien Giang province.

Remarks. This species differs from *H. histrix*, in having a shorter snout (45% of head length compared to 56%) and shorter spines. It is morphologically quite variable and may encompass a complex of

(a)

Male   Female   Dorsal

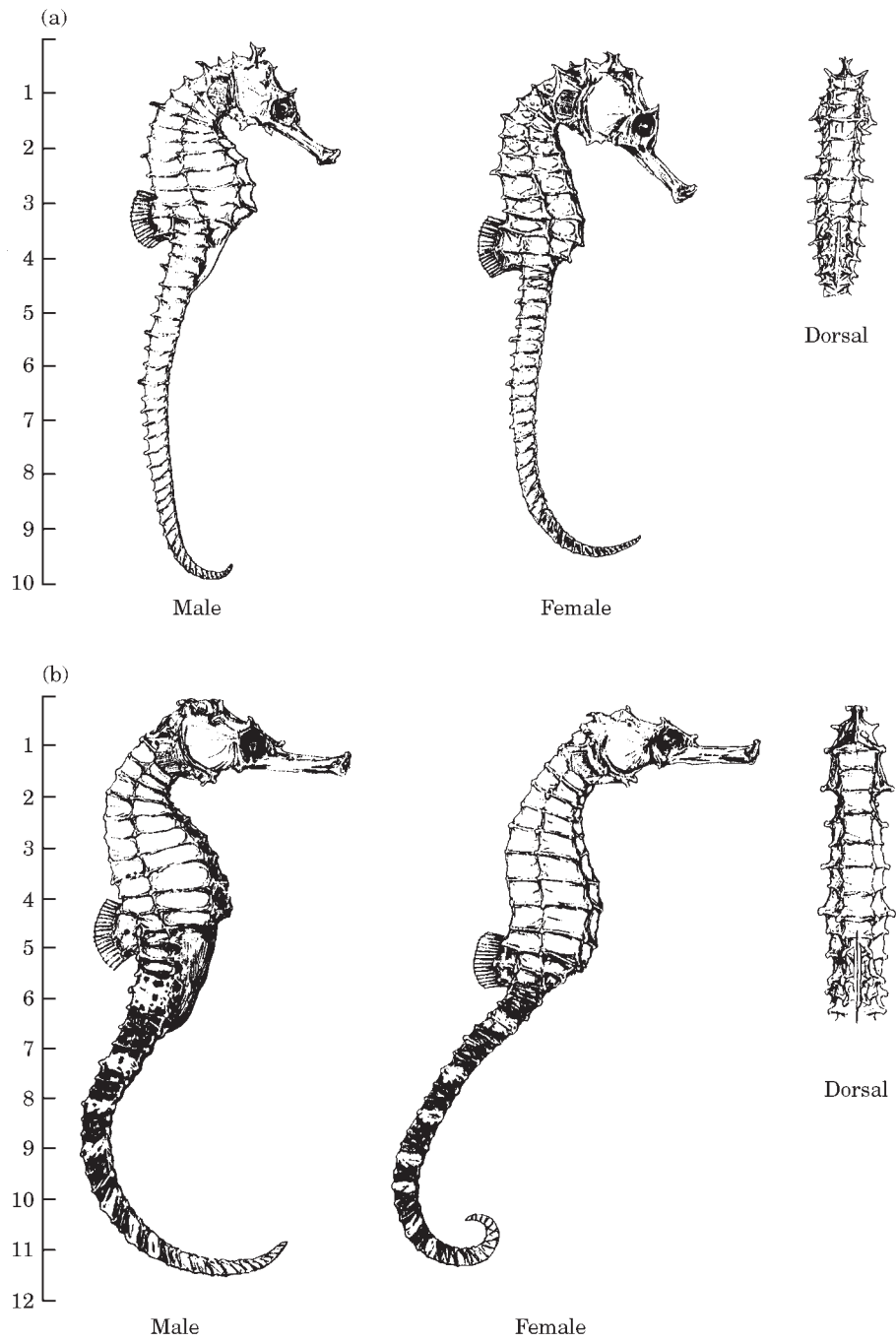(b)

Male   Female   Dorsal

Figure A3. Vietnamese seahorse species: (a) *H. spinosissimus*, (b) *H. comes*. Illustrations from left to right show: male, female, dorsal view of trunk (female). Scale bar is marked in cm.
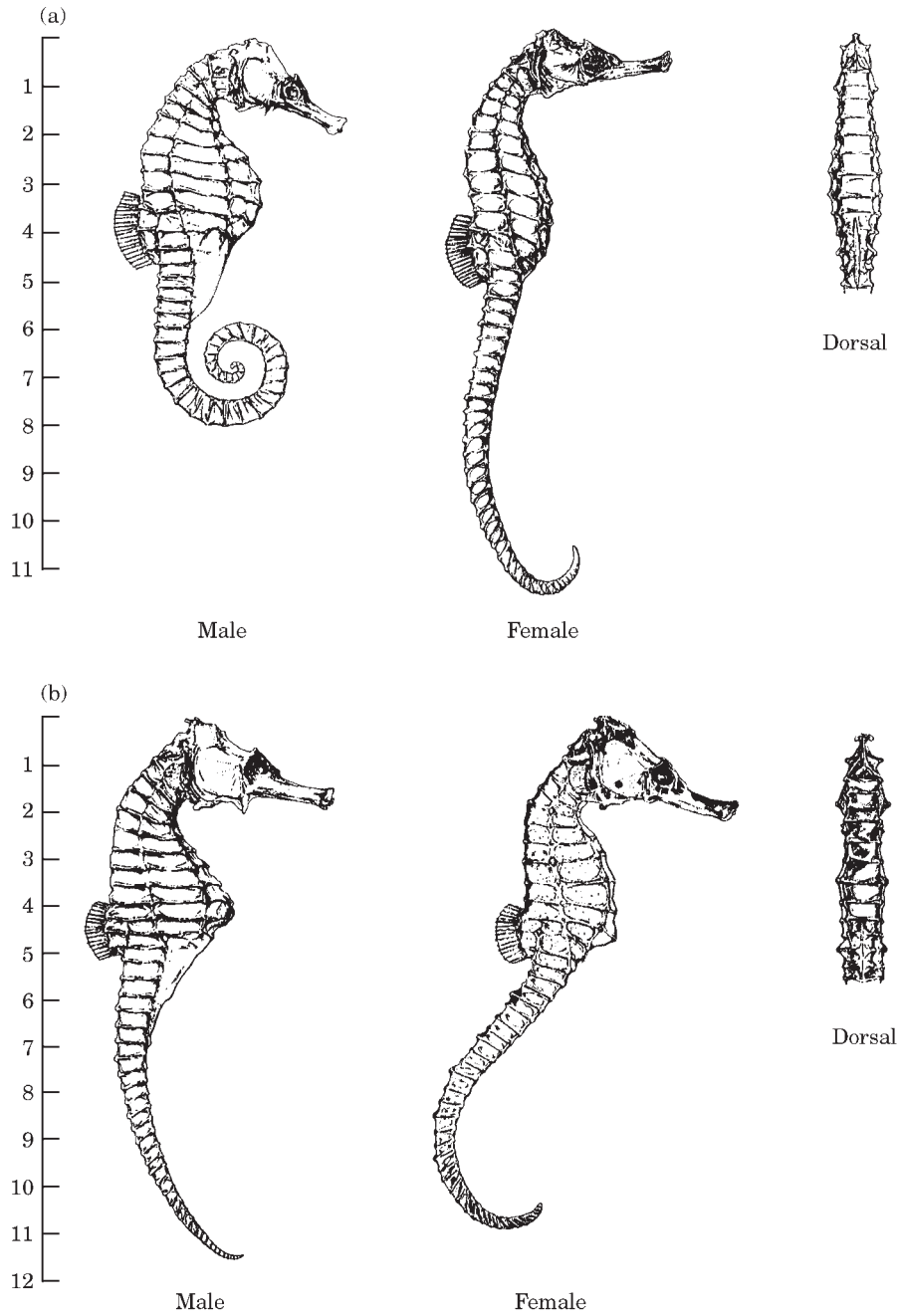
Figure A4. Vietnamese seahorse species: (a) *H. trimaculatus*; (b) *H. kuda*. Illustrations from left to right show: male, female, dorsal view of trunk (female). Scale bar is marked in cm.
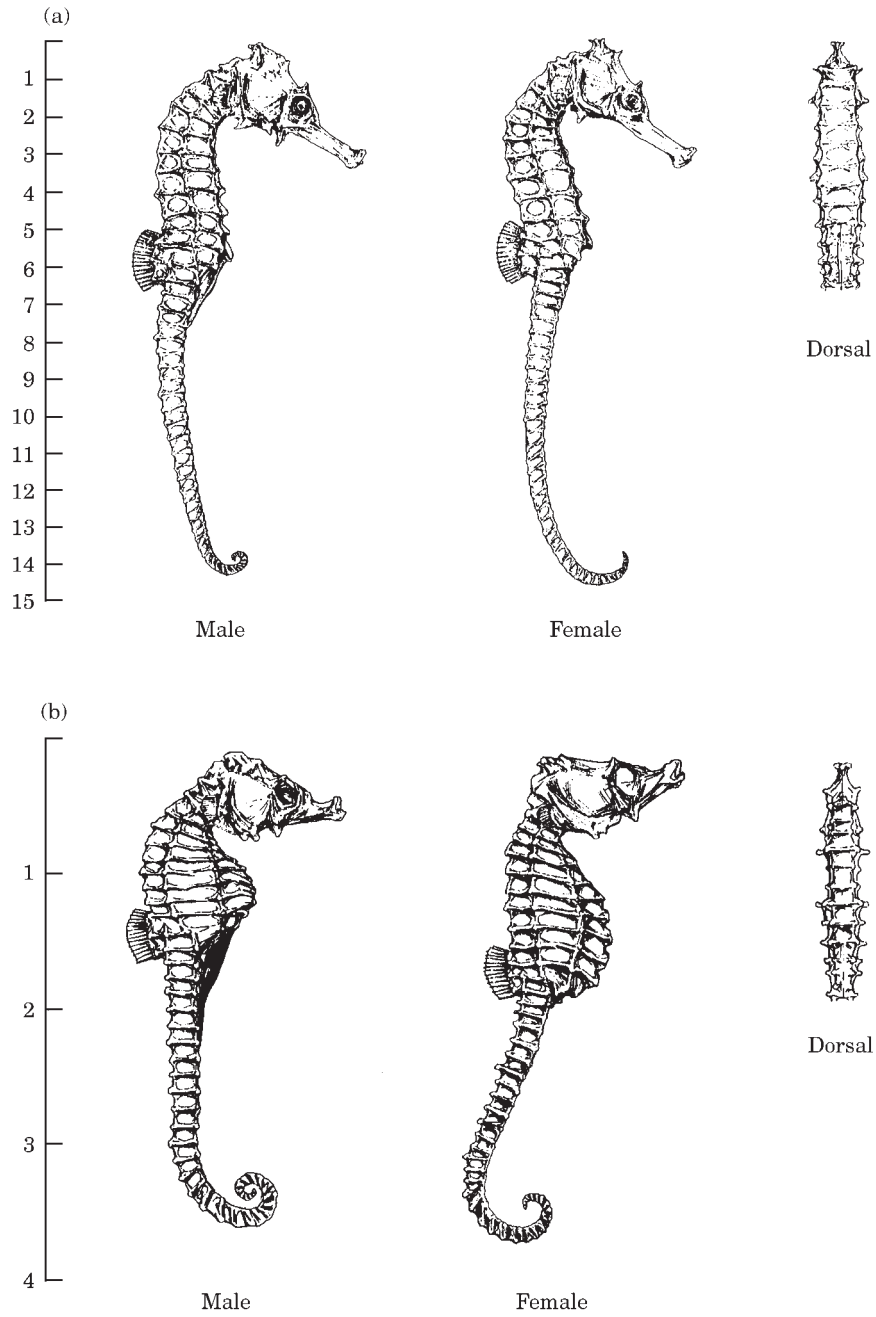
(a)

(b)

Figure A5. Vietnamese seahorse species: (a) *H. kelloggi*; (b) *H. mohnikei*. Illustrations from left to right show; male, female, dorsal view of trunk (female). Scale bar is marked in cm.
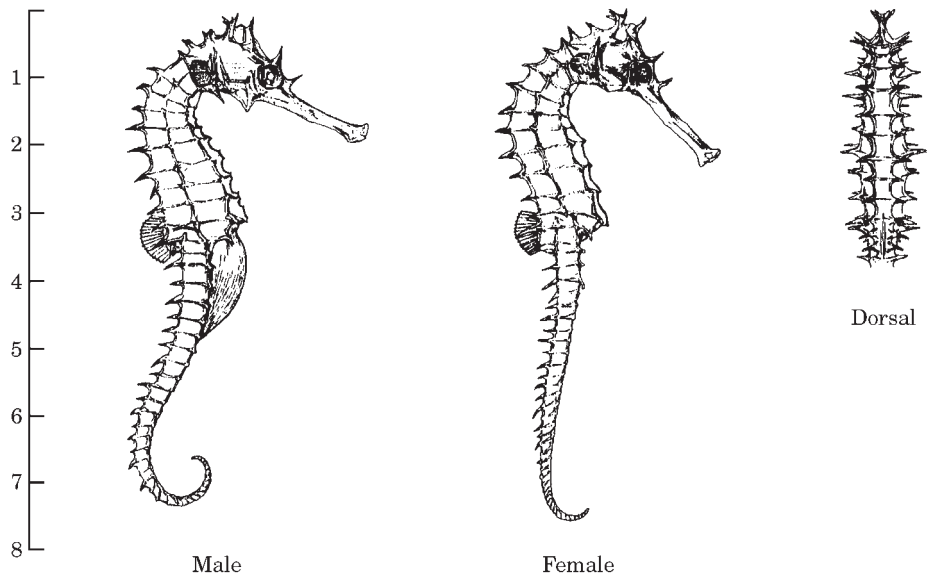
Figure A6. Vietnamese seahorse species: *H. histrix*. Illustrations from left to right show: male, female, dorsal view of trunk (female). Scale bar is marked in cm.

species. The type specimen of *H. erinaceus* has only 34 tail rings and is not considered a synonym of *H. spinosissimus*.

Morphotype B—H. comes Cantor 1850 (*Fig. A3b*)

Materials. Seven females, 1 male (no further males were available).

Type specimen examined. H. comes Cantor 1850: BMNH 1982.6.17.9 (from Pinang, Malaysia).
D 18; P 16–17(17); Rings 11 + 35

Description. Snout length about equal to length of rest of head. Coronet low with 5 rounded knob-like points. All junctions of body ridges surmounted by knob-like tubercles of approximately equal size. Double cheek spines bordering throat at base of cleithral ring and usually double spines above the eye.

Colour. Black, trunk with white blotches, tail with white transverse bands, tubercles with white subteminal band (may be muted and blotchy patterns may not be visible in dark specimens).

Standard length. 141–171 mm.

Habitat. Unknown (probably coral reef).

Inferred distribution. Khan Hoa, rare.

Morphotype C—Hippocaupus trimaculatus Leach 1814 (*Fig. A4a*)

Materials. Ten females, 10 males.

Type material examined. H. trimaculatus Leach 1814: BMNH 1982.6.17.43(2); 1982.6.17.44–45 (2) (from 'China Sea'); *H. manadensis* Bleeker 1856: RMNH 7257 (from Manado, Celebes, Indonesia); *H. kampylotrachelos* Bleeker, 1854b: RMNH 7256 (from Priaman, Sumatra, Indonesia).

D 19–21(20); P 17–19(18); Rings 11 + 39–42(41)

Description. Snout shorter than rest of head. Coronet barely raised above the line of the arc of the head but merely visible as five tiny spines directed backwards. No spines on junctions of body ridges.

Prominent sharp recurved spines bordering throat at base of cleithral ring, and also above orbit. Black spots often present on dorsolateral surface of 1st, 4th and 7th trunk rings. These are more visible in males and may be difficult to discern in dark coloured specimens.

Colour. Light yellow, brown.

Standard length. 96–160 mm.

Habitat. Gravel and sand.

Inferred distribution. Quang Ninh to Kien Giang.

Remarks. *H. kampylotrachelos* Blkr and *H. manadensis* Blkr are considered to be junior synonyms of *H. trimaculatus* Leach.

Morphotype D—H. kuda Bleeker 1852 (Fig. A4b)

Materials. Seven females, 7 males, 1 probable juvenile (not included in analysis).

Type specimens examined. *H. kuda* Bleeker 1852: RMNH 5167 (30 in jar labelled '*H. guttulatus* –probably includes the type specimen of *H. kuda*'), BMNH 1867.11.28.360 (from Singapore); *H. melanospilos* Bleeker 1854d: BMNH 1867.11.28.362 (from Ambon, Indonesia); *H. rynchomacer* Dumeril 1870: MNHN 5987–5991 (syntypes) (from 'Mer d'Indies, China, Singapore, Cochin China'); *H. barbouri* Jordan & Richardson 1908: CAS 120205 (paratypes) (from Cuyo, Philippines); *H. maculatus* Heckel (no date): RMNH 1532 (no locality); *H. aterrimus* Jordan & Snyder 1902: CAS 106516 (from Yaeyama, Ryukyu Islands, Japan); *H. polytaenia* Bleeker 1854c: BMNH 1867.11.28.361 (from Larantuka, Flores, Indonesia).

D 17–18(17); P 16–17(16); Rings 11 + 35–37(36)

Description. Snout shorter than rest of head. Coronet low, angled backwards with rounded top, and 5 blunt bulging points. Tubercles on body angles poorly developed giving this species a relatively smooth appearance. Males deeper bodied than females, often with distinct keel.

Colour. Dark black or brown, or pale yellow with tiny dark spots on body, larger blotches on face and darker dorsal surface.

Standard length. 124–168 mm (juvenile 97 mm).

Habitat. Estuaries, though may also be found in the open sea.

Inferred distribution. Thua Thien, Quang Nam-Da Nang, Khanh Hoa and Binh Thuan provinces.

Remarks. We have been conservative and retained the name *H. kuda*, which is currently in use in Vietnam for Morphotype D, pending a complete revision of the 'kuda' species complex. Not all proposed synonyms of *H. kuda* were examined in this study, and the species listed above should not necessarily be inferred to be synonymous.

Morphotype E—H. kelloggi Jordan & Snyder 1902 (Fig. A5a)
Materials. Six females, 6 males, 2 possible juveniles (included in PCA as females).

Type specimen examined. *H. kelloggi* Jordan & Snyder 1902: CAS 106521 (from Japan).

D 18–19(18); P 18–19(18); Rings 11 + 40–41(40)

Description. Snout length about equal to rest of head. Narrow, straight trunk. Coronet significantly raised above the line of the arc of the neck with 5 distinct spines. Rounded spines on junctions of body ridges. Trunk rings very thick and solid-looking. Younger specimens tend to have sharper spines. Long downturned rounded cheek spines bordering throat. Prominent spine above eye.

Colour. Pale yellowish.

Standard length. 162–288 mm (juveniles 127 and 131 mm).

Habitat. Unknown.

Distribution. Quang Nam-Da Nang, Khanh Hoa and Binh Thuan provinces.

Morphotype F—H. mohnikei Bleeker 1854 (*Fig. A5b*)

Material. Two females, 2 males.

Type specimens examined. H. mohnikei Bleeker 1854: RMNH 7259 (3) (from Japan); *H. japonicus* Kaup 1856: RMNH 1540; RMNH 5319 (5) (from Japan).

D 15; P 12–14(13); Rings 11 + 30, 35–36(36)

Description. Snout very short, about a quarter of total head length. Coronet raised with four slight points. Blunt, low spines on junctions of body ridges; those on 1st, 4th, 7th and 11th trunk ring and 4th, 7th and 10th tail rings are slightly more prominent. Double cheek spines bordering throat.

Colour. Dark brown or black.

Standard length. 54–57 mm.

Habitat. Estuaries.

Inferred distribution. Quang Tri and Khanh Hoa provinces. Rare.

Remarks. Morphotype F appears to be genetically distinct from *H. mohnikei* from Japan (unpublished data). However, this is based on a single specimen only, and until we have more information we shall retain the name *H. mohnikei* for Morphotype F.

Morph G—H. histrix Kaup 1856 (Fig. A6)

Materials. Four females, 4 males, 1 probable juvenile (not included in analysis).

Type specimens examined. H. histrix Kaup 1856: MNHN A.906, RMNH 1537 (from Japan).
D 17; P 18; Rings 11 + 34–37(35)

Description. Snout much longer than rest of head. Coronet with 4–5 long spines. Very long spine immediately in front of coronet. Also long single spine above eye and between orbits, and prominent double spine below eye. Very long, extremely sharp, often dark-tipped, spines on all junctions of body ridges; those on the 1st, 4th, 7th and 11th trunk ring and 1st, 4th, 6th, 8th, 10th and 12th tail ring still longer. Long sharp cheek spines (never double) bordering throat at base of cleithral ring. Base of dorsal fin very short.

Colour. Bright yellow or yellow.

Standard length. 90–134 mm (juvenile 81 mm).

Habitat. Unknown.

Distribution. Khanhoa and Ninthuan provinces.

Remarks. This is *not* the *H. histrix* of Weber & Beaufort (1922) which was a mis-identification (personal observation of the latter specimens in ZMA).