Phylogenetic relationships of the commercial marine shrimp family Penaeidae from Persian Gulf

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Abstract

Phylogenetic relationships among all described species (total of 5 taxa) of the shrimp genus Penaeus, were examined with nucleotide sequence data from portions of mitochondrial gene and cytochrome oxidase subunit I (COI). There are twelve commercial shrimp in the Iranian coastal waters. The reconstruction of the evolution phylogeny of these species is crucial in revealing stock identity that can be used for the management of fish industries in Iran. Mitochondrial DNA sequences were used to reconstruct the phylogeny of the *Penaeus* species of marine shrimp. For this purpose, DNA was extracted using phenol- chloroform well as CTAB method. The evolutionary relationships among 5 species of the shrimp genus *Penaeus* were examined using 610 bp of mitochondrial (mt) DNA from the cytochrome oxidase subunit I gene. Finally the cladograms were compared and the resulting phylogenetic trees confirmed that the Iranian species originated from the Indo-west pacific species. The Iranian species, which were not grouped with the other Penaeid taxa, seem to always form a sister-clade to the Indo-west pacific species with strong bootstrap support of 100%. Although the Iranian species + Western Hemisphere clade is paraphyletic in our gene tree, the bootstrap support is high. However, we still lack any comprehensive and clear understanding of phylogenetic relationships in this group.

Keywords: Mitochondrial DNA, Penaeidae, Persian Gulf, Phylogeny

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Introduction

The Penaeid shrimp form a diverse group of marine decapods with over 400 species globally distributed and inhabiting both shallow waters and abyssal zones below 5000 m (Pérez Farfante and Kensley, 1997). The Penaeid industry has grown rapidly through the last 30 years and the dominant species are *Penaeus monodon* (Fabricius 1798), *P. vannamei* (Boone 1931), *P. chinensis* (Osbeck 1765) *P. stylirostris* (Stimpson 1874), *P. japonicus* (Bate 1888), *P. merguiensis* (De Man 1888) and *P. indicus* (H. Milne Edwards 1837).

Shrimp of the genus *Penaeus* (Crustacea: Decapoda) are the most abundant group of marine groups in tropical and subtropical waters around the world. Due to the lack of research in Iran, to resolve phylogeny relationships within this group is the main reason to perform this study. It is important to manage wild stocks differently than it was done for *P. monodon. P. merguiensis*. For example, *P. indicus*, *P. japonicus*, and *P. vannamei* together can be easily be matured in captivity without eyestalk ablation (Bailey-Brock and Moss, 1992). Unfortunately, Chaitiamvong and Supongpan (1992) tolled the existence of over 50 *Penaeid* species in Thai waters, four of them (*P. merguiensis*, *P. indicus*, *P. silasi*, and *P. penicillatus*) are considered morphologically similar species. Taxonomic difficulties about the external morphology of *P. merguiensis* has been reported (Chong and Sasekumar, 1982).

As is known, mtDNA is a useful maternal marker because of its matrilineal mode of inheritance. In animals, mtDNA genes such as COI are particularly suitable for the determination of intraspecific genetic diversity owing to a high evolutionary rate found in this maternally transmitted genome (Avise *et al*., 1987). Therefore it has been widely used as a genetic marker to assess the phylogeography and phylogeny within numerous species and genera (Baldwin *et al*., 1998; O'Grady *et al*., 1998; Funk, 1999; Reed and Sperling, 1999; Masta, 2000; Walton *et al*., 2000). Baldwin *et al*. (1998) examined 13 different species of *Penaeus* using 558 bp of mitochondrial DNA from COI and was able to successfully divide the genus into two groups (Burkenroad, 1934). Mitochondrial DNA (mtDNA) sequences have proved extremely useful in elucidating phylogenetic relationships among many crustacean groups (Cunningham *et al*., 1992). Mitochondrial large subunit ribosomal RNA (16S rRNA) and cytochrome c oxidase subunit I (COI) genes have been particularly helpful in analyzing crustacean phylogeny at the species level (Chu *et al*., 2003). Baldwin *et al*. (1998) examined the phylogenetic relationships of 13 *Penaeus s.l.* species based on COI sequences. The evolutionary partitions resolved do not support the subgenus division but are concordant with biogeographic

groupings within the genus. In particular, their data show that *Litopenaeus* and *Farfantepenaeus* are not valid phylogenetic groupings, but are paraphyletic (Baldwin *et al*., 1998).

To date, 28 species are recognized in Penaeus (Dall *et al*., 1990). There have been two studies so far based on 16S rRNA or combined 16S and COI sequences of the superfamily Penaeoidea in which 16 species (Vázquez-Bader *et al*., 2004) and 11 species (Quan *et al*., 2004) of the Penaeidae were included. Results from the studies, however, question the monophyletic status of the family Penaeidae. Voloch *et al.* (2005) examining the phylogenetic relationships of the Penaeidae by analyzing 16S and COI sequences of 39 species of the Penaeidae based on existing data (Tong *et al.*, 2000; Maggioni *et al.*, 2001; Lavery *et al.*, 2004; Quan *et al.*, 2004). Since *Penaeus s.l.* shrimp are remarkably well-known and are of great economic importance separating them into six genera can produce significant impact on both scientific and commercial practices.

In recent years there have been a variety of phylogenetic researches on *Penaeus* species using genetic markers to solve questions regarding their taxonomy and evolution. Unfortunately, all previous analyses have been severely constrained by data limitations in the number of species and the number of genetic characters. Overall there is still considerable doubt about the true relationships among all *Penaeus* species, and we still lack any comprehensive and clear understanding of phylogenetic relationships in this group.

The aim of this study was the molecular identification of 5 commercial shrimp species of the Persian Gulf and Oman Sea, using a DNA sequencing analysis of cytochrome oxidase subunit I (COI). In the present study, the mitochondrial cytochrome oxidase subunit I gene was chosen for analysis as it has proven useful in studying phylogenetic relationships at the species level in many arthropods (Howland and Hewitt, 1995).This study provides new COI sequences from 5 species, allowing COI phylogenetic analysis of almost the entire group. The Indian-Pacific has the most diverse species, with about five times of that found in the Atlantic, showing the region of the origin for this widespread genus (Dall *et al.*, 1990). The second goal of this study was to determine whether the Indo-West Pacific is the origin of the genus. Finally, the material presented here provides a phylogenetic framework for management of these important natural resources. Bernatchez (1995), reported wildlife management programs that depend on an understanding of the phylogenic relation. Marine shrimp of the superfamily Penaeoidea represent approximately a third of the world's commercially important shrimp species and account for over 80% of the wild catch. In addition, members of the

genus *Penaeus* represent over 90% of the cultured species worldwide.

Materials and methods

Collection of materials

Five species of commercial shrimp belonging to the family Peneaidae, were collected by fishing trawler vessel Ferdows II from northern parts of the Persian Gulf and preserved in 95% ethanol. The sampled species are the following: *Parapenaeopsis stylifera* (H. Milne Edwards, 1837 [in H. Milne Edwards, 1834-1840]), *Metapenaeus stebbingi Nobili*, 1904, *M. stridulans* (Alcock, 1905). *P. semisulcatus*, *P. (Fenneropenaeus) merguiensis*. The sampling sites and the number of specimens examined are listed in Table 1.

The Chinese mud shrimp, *Solenocera koelbeli* De Man, 1911, of the family Solenoceridae (Decapoda, Dendrobranchiata, Penaeoidea), was involved in the study that was chosen as an outgroup (Table 1). Five individuals of each species were used for molecular sequence analysis and the species were identified using several shrimp species keys (Dall *et al*., 1990) and they were also identified by the researcher (Mohsen Safaiee), who is an experienced shrimp taxonomist.

Table 1: Details of specimens and sequences used in this study.

Species	N	Location	GenBank Accession Nos.a
P. (Farfantepenaeus) aztecus	1	Gulf of Mexico, USA	AF279834
P. (Farfantepenaeus) brasiliensis	1		AF029393[1]
P. (Farfantepenaeus) californiensis	1		NC 012738
P. (Farfantepenaeus) duorarum	1	Gulf of Mexico, USA	AF279835
P. (Farfantepenaeus) notialis	1		X84350
P. (Farfantepenaeus) paulensis	1		AF029392[1]
P. (Farfantepenaeus) subtilis	1		AF248559[4]
P. (Fenneropenaeus) chinensis	1	Zhujiang estuary, China	AF279836
P. (Fenneropenaeus) indicus	1	Bonaparte Gulf, W. Australia	AF279837
P. (Fenneropenaeus) merguiensis	4	Moreton Bay, Australia Persian Gulf	AF279838 KR261589(40), KR261590(41), KR261591(43)
P. (Fenneropenaeus) penicillatus	1	Zhujiang estuary, China	AF279839
P. (Fenneropenaeus) silasi	1	Singapore	AF279840
P. (Litopenaeus) setiferus	1	Gulf of Mexico, USA	AF279841
P. (Litopenaeus) stylirostris	1		AF2550572 S65261[6]

Continued Table 1:

Species	N	Location	GenBank Accession Nos.a
P. (Litopenaeus) vannamei	1	Hawaii, USA	AF279842
P. (Marsupenaeus) japonicus	1	Hong Kong	AF279832
P. (Melicertus) canaliculatus	1	Taiwan	AF279843
P. (Melicertus) kerathurus	1	Spain	AF279844
P. (Melicertus) latisulcatus	1	Hong Kong	AF279845
P. (Melicertus) longistylus	1	North Queensland, Australia	AF279846
P. (Melicertus) marginatus	1	Taiwan	AF279847
P.(Melicertus) plebejus	1	Moreton Bay, Australia	AF279848
P. (Penaeus) esculentus	1	Moreton Bay, Australia	AF279849
P. (Penaeus) monodon	1	Hong Kong	AF279833
P. (Penaeus) semisulcatus	4	Hong Kong	AF279831
		Persian Gulf	KR261586(30), KR261587(33), KR261588(34)
P. stylifera	3	Persian Gulf	KR261592(20), KR261593(22), KR261594(23)
M. stebbingi Nobili	1	Persian Gulf	KR261595(2)
M. stridulans	3	Persian Gulf	KR261583(112), KR261584(113), KR261585(114)
Solenocera koelbeli		Hong Kong	AF105049

Sources sequences: [1]Baldwin *et al*. (1998); [2]Maggioni *et al*. (2001); [3]Garcia-Machado *et al*. (1996), [4]Gusm~ao *et al*. (2000);[5] Guti_errez-Mill_an *et al*. (2002); [6]Palumbi and Benzie (1991).

DNA extractiogarcian, PCR amplification and sequencing

Muscle tissues of pleopods, antenna and preiopods were removed from exoskeleton, preserved in 95º ethanol (Ovenden *et al*., 1997), and transferred to the laboratory. Three individuals of each species were used for molecular analysis. Samples of muscle from pleopods (10–15 mg) were used for extraction of total DNA using proteinase-K digestion and either phenol/chloroform and CTAB method extraction. Total genomic DNA was individually extracted from frozen muscle of shrimp.

The tissue was cut into small pieces and was homogenized in an appropriate volume of the extraction solution (10 mM Tris–HCl, pH 7.5, 100 mM EDTA, 1% SDS, and 1 Ag/ml proteinase K). The homogenate was incubated at 37 °C, 1 h, and 55 °C overnight. The DNA was extracted using phenol/chloroform/isoamyl alcohol (25:24:1) and recovered by ethanol precipitation. DNA concentrations were spectrophotometrically determined (Sambrook *et al*., 1989).

Total DNA was isolated using standard phenol-chloroform extraction protocol (Hillis and Moritez, 1990). Segments of mitochondrial genes, 610 bp fragment of cytochrome c oxidase subunit I (COI) gene were amplified from total DNA by polymerase chain reaction (PCR) (Saiki *et al*., 1988) with conserved primers: L-CO1490 and H-CO2198 for COI (Folmer *et al*., 1994).

PCR amplifications were conducted in a Amersham Pharmacia Biotechthermal cycler TC 341 using a 5 min pre-denaturation at 95 ºC followed by 30 cycles of 1 min at 95 ºC, 1 min at 40-46 ºC, 90 s at 72 ºC and a final 5 min at 72 ºC, were used to amplify 2 Ml of the DNA extract (undiluted or diluted 10_ in ddH2O), 2.5 Ml of Mg_2 free buffer (Promega), 0.7 Ml $MgCl_2$, 0.3 Ml of each primer, 0.45 Ml of dNTPs, 0.3 Ml of Taq polymerase (Promega), and ddH_2O.

In all PCR amplifications, negative controls consisting of template-free reactions were used to detect contamination. The size and quality of PCR products were evaluated on 1.5 % agarose gels.

Phylogenetic analysis

Alignment and Phylogenetic Analyses

Alignments of the sequences for this gene region were analyzed in a two-step process. First they were created with Clustal W (Higgins and Sharp, 1988) and then adjusted by eye to make the final alignments. The 38 taxa for COI alignment were subjected to maximum parsimony, and maximum likelihood (Felsenstein 1981) as applied in version 4.0 b10 of PAUP* (Swofford, 1999). Bayesian analyses was conducted using MrBayes v 3.0b4 (Ronquist and Huelsenbeck, 2003).

The construction of phylogenetic hypotheses from the data set was done using the maximum parsimony (MP), and maximum likelihood (ML) methods. For MP, we obtained the most parsimonious tree or trees with tree bisection-reconnection (TBR) branch-swapping heuristic searches in PAUP⋆ in which, all characters were equally weighted and the starting trees were obtained by 100 random stepwise additions. Nodal support was estimated by calculation of non-parametric bootstrap (1000 pseudo-replicate, 10 random addition) proportions (Felsenstein, 1985) and decay indices (Bremer, 1994) using PAUP⋆ PAUP* v 4.0b10. The Model Test (Posada and Krandall, 1998) was used to determine the optimal model of nucleotide substitution in the ML analysis. Because of the number of taxa involved and computational time requirement, branch support for the best fitting tree from ML analyses was assessed using 100 bootstrap replicates, *Solenocera koelbeli* was also included in all analyses.

The consensus phylogenetic trees generated by these three methods are shown in Fig. 1.

Results

The sequences used for phylogenetic analyses were 610 bp COI. Including the out group species and all the new sequences from the current study have been deposited in GenBank (see Table 1 for accession numbers).

The COI sequences of *P. semisulcatus* and *P. merguiensis* from Lavery *et al*. (2004) were not very different from the sequences of *P. semisulcatus* and *P. merguiensis* gained in the present report.

The sequence analyses were based on 610 bp for COI. All species were collected from Persian Gulf the species were identified using several shrimp species keys.

We decided to use sequences of Indo-West Pacific species and Western hemisphere from Lavery *et al*. (2004) for the subsequent phylogenetic analyses. Using Model test (Posada and Crandall, 1998), the best-fitting models of substitution for the COI data was the transversion model with the general time reversible model with a proportion of invariable sites and with a gamma distribution (GTR + I +G).

Fig. 1 shows the best-fitting tree topology based on maximum likelihood analysis, Bayesian and parsimony analysis.

In all the trees data approach, there was very strong support (bootstrap P 100) for the monophyletic status of species (Fig. 1). There was also strong support for the monophyletic status of the indo-pacific speices and Iranian species, although the presence of *P. chinensis* in the monophyletic clade with *Farfantepenaeus* and *Litopenaeus* subgenera weakened the support slightly (bootstrap P 54+69).

Iranian shrimp is in the monophyletic clade to indopacific group of high bootstrap values (bootstrap P 100) in all the analyses.

M. stridulans and *P. stylifera* were most closely related with *P. merguiensis* as the sister taxon. *P. semisulcatus* then joined this subclade with M. stebbingi as the most outlying sister taxon.

The two species, *P. merguiensis* and *P. semisulcatus*, were always placed in the different clade in all analyses with relatively strong bootstrap support (100 by parsimony analysis, 84 by maximum likelihood and Bayesian).

M. stebbingi and other Iranian species were always placed in the different monopyletic groups in all analyses with relatively strong bootstrap support (100 by all analysis).

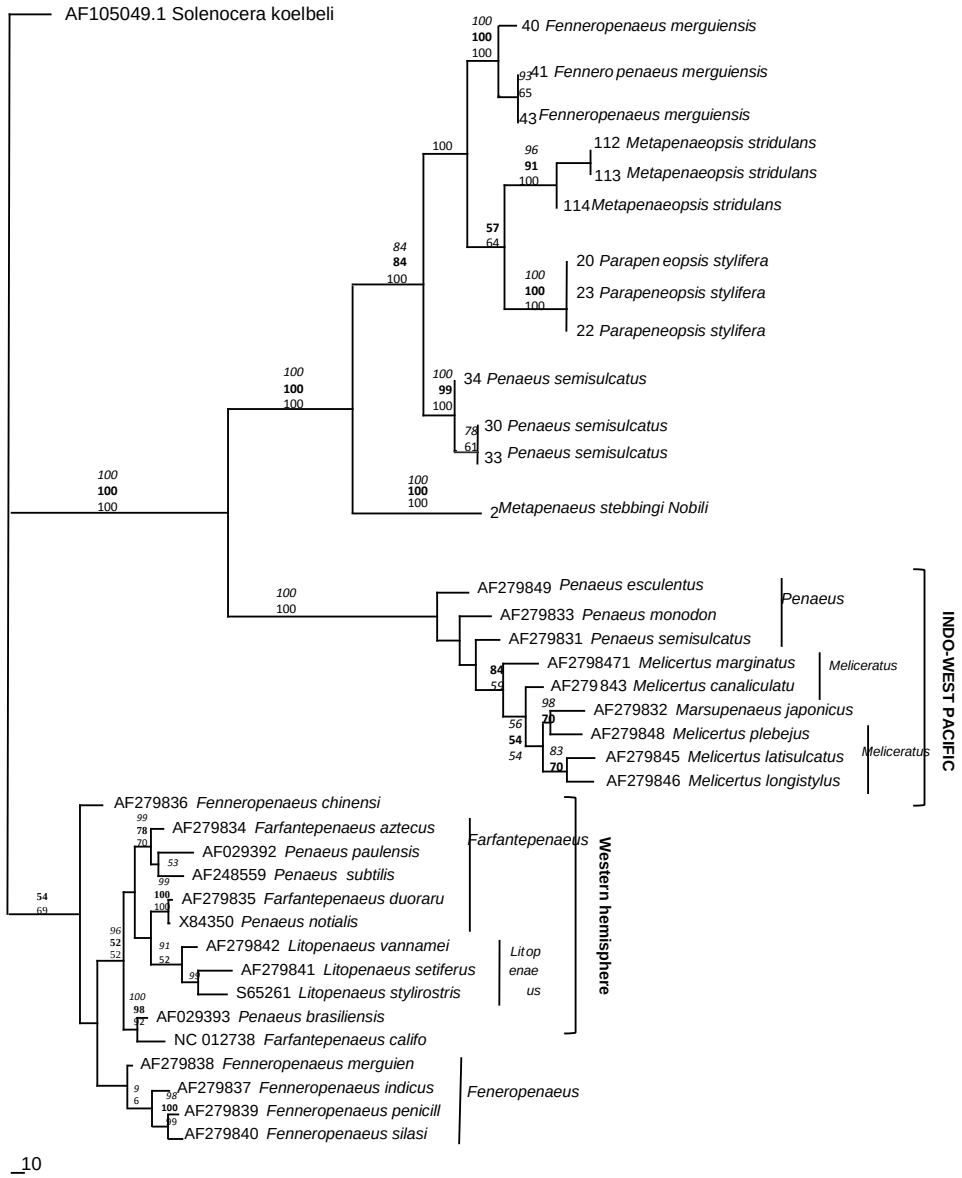

Figure 1: Numbers above branches indicate bootstrap values from Bayesian analysis (in italics), Parsimony analysis (normal text), and Maximum Likelihood analysis (in bold). Branches without bootstrap numbers indicate that the bootstrap values are not existent in the trees.

Bootstrap support of the Western hemisphere group was lower than that of the Indo-west Pacific. Among the Indo-Pacific species there is robust support were the relationships between *Marsupenaeus japonicas* and *Melicertus plebejus*, and *Melicertus latisulcatus* of within *Melicertus longistylus*, but parsimony, Maximum likelihood and MrBayes failed to resolve relationships among the remaining Indo-Pacific species, *P. monodon* and *P. semisulcatus*.

In all three methods of analysis *P. kerathurus*, the sole eastern Atlantic species which was grouped strongly (indicated by high bootstrap support) with *P. japonicus* and *P. canaliculatusto* formed a distinct clade. The eastern Pacific species *P. vannamei* and *P. stylirostris* show close relationships.

Among the Iranian species the monophyly of the clade including *F. merguiensis* and *M. stridulans*, *P. stylifera* is strongly supported by parsimony bootstrap (100).

Discussion

Our study reveals the utility of COI genes in the reconstruction of Penaeus phylogeny. The combinations of almost all species from the whole genus has allowed the present study to show a relatively clear and well-supported pattern of phylogenetic relationships among the major groups of *Penaeus* species in Iran. The Penaeidae evolutionary history is elongated (Dall *et al*., 1990). The family has highest diversity in the Indo-West Pacific region. About 200 species are known and these have generally been grouped into 17 genera (Dall *et al*., 1990) although, the recent studies of the *Penaeus* genera, established 26 genera in the family Penaeidae. Pérez Farfante and Kensley (1997). While the taxonomy of Penaeids has been generally studied, research on the complete phylogenetic relationships between the genera and species is limited. In Iran the lack of phylogenetic relationships of these studies brief these relationships.

The present study compares the phylogenetic hypotheses of the Penaeidae derived from new molecular data from Iran on Penaeidae, to the previous schemes, and analyzing 610 bp of aligned mitochondrial COI gene sequences using, maximum parsimony, maximum likelihood and Bayesian inference methods yields similar topologies (Fig. 1) with two major clades.

Lavery *et al*. (2004) used 26 16S partial sequences to study the phylogenetic relationships of the family Penaeidea. We tried to re-analyze the sequences of the Penaeidae (with outgroup taxa) utilized by them and found that the Penaeid taxa formed two major clades, which are consistent with our own results. The Iranian species, which were not grouped with the other Penaeid taxa, seem to always form a sister-clade to Indo-west pacific species with strong bootstrap support of 100%. We found out that the origin of Iranian species is the Indo-west pacific species. The molecular data support an Indo-West Pacific origin of the genus with a single relatively recent colonisation of the Western Hemisphere, and the subsequent subdivision into two clades prior to the emergence of the Panamanian isthmus (Lavery *et al*., 2004).

The results of Lavery *et al*. (2004) that the subgenera *Farfantepenaeus* and *Litopenaeus* are not paraphyletic are in accordance with those in our study,

however, they differ from those given by Baldwin *et al*. (1998) and Gusmao *et al*. (2000). The key conclusion from a previous molecular study indicating that the subgenera *Farfantepenaeus* and *Litopenaeus* are paraphyletic was rejected. *Litopenaeus* species express a clade, with close relationship to *Farfantepenaeus*, both of which clade to the sister clade of *Fenneropenaeus*. Similar results for the phylogenetic relationships among the Penaeid shrimp were obtained in previous studies. It is based on protein-coding genes (Shen *et al*., 2007). Parsimony bootstrap and ML strong statistical bootstrap support the monophyly of all Penaeids that was observed across the analysis (Shen *et al*., 2007).

Although the Iranian species + Western Hemisphere clade is paraphyletic in our gene tree, the bootstrap support is very high. Maximum parsimony tree, Maximum Likelihood tree and Bayesian tree show a similar topology for the Penaeid shrimp (Fig. 1). Parsimony bootstrap and ML strong statistical bootstrap support for the monophyly of all Iranian Penaeid were observed across the analysis. *P. stylifera* define a clade, with close relationship to *M. stridulans*, and both clade to the sister group of *Penaeus*, *F. merguiensis*, and *P. semisulcatus*. *P. stylifera* and *F. merguiensis*, as were defined, are apparently monophyletic.

Information about *M. stridulans* and *M. stebbingi* and *P. stylifera* genes is very limited, currently only one sequence is found in GenBank for *P. stylifera* and no sequence of *M. stridulans* and *M. stebbingi* is found. The current results on these three mitogenomes can be used to obtain more important information in later studies.

The phylogenetic relationships of the decapods are as contentious as ever. Despite the limited number of taxa tested in this study, studies with more taxa involving the complete mitochondrial genomes of this family are desirable to understand the phylogeny of the Penaeidae. In summary, our molecular phylogeny has clarified the relationships within the genus Peneaidae family in Iranian coastal waters. Our phylogenetic results represent the first step toward understanding the pattern of speciation in Penaeidae in Iran, the basis of molecular characters.

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