



RESEARCH NOTE

Supraspecific taxonomy in the Vertiginidae (Gastropoda: Stylommatophora)

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The genus *Vertigo* Müller, 1774 consists of *c.* 100 species of terrestrial microsnails *c.* 1.5–3 mm in length with a rounded aperture and 0–6 (sometimes more) apertural lamellae at maturity. As currently defined, the genus is largely Holarctic in distribution with only a few Neotropical species being known (Pilsbry, 1948; Nekola & Rosenberg, 2013).

Consensus does not exist concerning supraspecific taxonomy of the genus. Pilsbry (1919, 1927, 1948) placed *Vertigo*, *Columella* Westerlund, 1878 and *Truncatellina* Lowe, 1852 in the family Pupillidae, subfamily Vertigininae. Based largely on anatomy, he differentiated these from the subfamily Nesopupinae, into which he placed 11 mostly tropical genera: *Bothriopupa* Pilsbry, 1898, *Campolaemus* Pilsbry, 1892, *Costigo* Boettger, 1891, *Cylindrovertilla* Boettger, 1880, *Lypopupa* Pilsbry, 1900, *Nesopupa* Pilsbry, 1900, *Pronesopupa* Iredale, 1913, *Ptychalaea* Boettger, 1889, *Pupisoma* Stoliczka, 1873, *Staurodon* Lowe, 1854 and *Sterkia* Pilsbry, 1898. Bouchet & Rocroi (2005) assigned *Vertigo* to the family Vertiginidae, which in their scheme comprised three subfamilies: Vertigininae, Nesopupinae (both largely as designated by Pilsbry, 1927, but with *Pupisoma* being moved to the Valloniidae) and Gastrocoptinae (including *Gastrocopta* Wollaston, 1878 and 10 related genera). The Gastrocoptinae have subsequently been assigned to the Chondrinidae (Pokryszko *et al.*, 2009).

Pilsbry (1919, 1948) recognized four subgenera within *Vertigo*: *Vertigo* s.s. and the monotypic *Angustula* Sterki, 1888, *Vertilla* Moquin-Tandon, 1855 and *Vertillaria* Pilsbry, 1919. He further divided *Vertigo* s.s. into several informal sections and groups. This treatment was followed until Turgeon *et al.* (1998) and Sysoev & Schileyko (2009), respectively, considered the group *Nearctula* Sterki, 1892 and the subgenus *Vertilla* to be of generic rank. Similar intrageneric division has been prominent within *Nesopupa*; e.g. Gittenberger & van Bruggen (2013) recognized nine genera originally proposed as sections by Pilsbry & Cooke (Pilsbry, 1919): *Afripupa*, *Cocopupa*, *Helenopupa*, *Indopupa*, *Infranesopupa*, *Insulipupa*, *Nesodagys*, *Nesopupa* and *Nesopupilla*.

To address these issues empirically, we have assembled and phylogenetically analysed DNA sequence data from the nuclear 28S ribosomal RNA (28S) and mitochondrial 16S ribosomal RNA (16S) genes. The species selected for study included the full conchological, biogeographic and ecological range of *Vertigo*, plus representatives of *Columella*, *Gastrocopta*, *Nearctula* and *Truncatellina*, and a variety of putative nesopupillids and other genera within the Pupillidae (*sensu* Pilsbry, 1927). We also included a representative sampling across the infraorder

Orthurethra as designated by Wade, Mordan & Clarke (2001). Among the analysed taxa, the following represent the type species of their respective genera: *Acanthinula aculeata* (Müller, 1774), *Chondrina avenacea* (Bruguière, 1792), *Cochlicopa lubrica* (Müller, 1774), *Columella edentula* (Draparnaud, 1805), *Helix pomatia* Linné, 1758, *Lauria cylindracea* (da Costa, 1778), *Leiostylia anglica* (Wood, 1828), *Nearctula californica* (Rowell, 1862), *Planogyra asteriscus* (Morse, 1857), *Pupilla muscorum* (Linné, 1758) *Pyramidula rupestris* (Draparnaud, 1801), *Solatopupa similis* (Bruguière, 1792), *Sterkia calamitosa* (Pilsbry, 1889), *Strobilops labyrinthica* (Say, 1817), *Vallonia costata* (Müller, 1774), *Vertigo pusilla* (Müller, 1774), *Vertilla angustior* (Jeffreys, 1830) and *Zoogenetes harpa* (Say, 1824). *Carychium tridentatum*, *Helix pomatia* and *Cornu aspersum* were used as comparative outgroups.

Archived GenBank sequences were used for 24 specimens, including data first reported by Armbruster *et al.* (2005) [AY546471], Dinapoli, Zinssmeister & Klusmann-Kolb (2010) [GU331954], Gaitan-Espitia, Nespolo & Opazo (2013) [JQ417194], Ketmaier *et al.* (2010) [[GU046389], Nekola & Rosenberg (2013) [KF214500, KF214496], Nekola *et al.* (2012) [JN941017, JN941032, JN941041, JN941044], Nekola, Coles & Bergthorsson (2009) [GQ921543], Wade, Mordan & Naggs (2006) [AY841284, AY841285, AY841286, AY841333], Wade *et al.* (2001) [AY014019, AY014020, AY014022, AY014023, AY014024, AY014025, AY014027, AY014028, AY014028, AY014030, AY014032, AY014033, AY014040, AY014148] and Weigand *et al.* (2013) [KC206171]. Sequences for the remaining 35 specimens (Table 1) were newly obtained. DNA extraction, purification, PCR amplification and sequencing were performed using previously published methods and primers (see Wade & Mordan, 2000; Nekola & Rosenberg, 2013). The amplified 28S region (Fig. 1) encompasses ITS-2, a region that cannot be aligned because of its intergeneric hypervariability. As a result, all sequence more than 284 bp upstream of the LSU2 primer of Wade & Mordan (2000) was excluded from analysis. The resultant analysed 28S amplicon ranged in length from 809 bp (*Acanthinula aculeata*) to 827 bp (*Columella edentula*). The entire 16S amplicon was used for analysis and ranged in length from 403 bp (*Cornu aspersum*) to 594 bp (*Truncatellina cylindrica*).

Sequences were aligned using ClustalX, with adjustment by eye. Mega v. 5.0 was used to conduct neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses separately for the 28S and 16S sequences. NJ analysis was based on maximum composite distance including transitions

Table 1. Collection information for specimens analysed in this study.

Taxon	Country	State or equivalent	County or equivalent	Site	Lat./Long.	Collection/ accession no.	16S GenBank acc. no.	28S GenBank acc. no.
<i>Afripupa bisulcata</i>	Ghana			Fetish Grove	5.3356 N, 0.0801 W	deWinter	KT008314	KT008353
<i>Bothriopupa tenuidens</i>	Costa Rica	Puntarenas		Saladera Lodge	8.6988 N, 83.3302 W	Tattersfield		KT008355
<i>Columella edentula</i>	France	Normandy	Calvados	La Vallée des Vaux	48.9446 N, 0.4678 W	BC00001	KT008310	KT008360
<i>Leiostyla anglica</i>	UK		Cumbria	Meathop Cliff Seep	54.2066 N, 2.8729 W	JCN	KT008308	KT008363
<i>Nearctula californica</i>	USA	California	Monterey	Moss Landing Beach	36.8095 N, 121.7880 W	JCN13934	KT008315	KT008349
<i>Nearctula rowelli</i>	USA	California	Placer	Auburn	38.9072 N, 121.0520 W	JCN17232	KT008327	KT008347
<i>Nesopupa newcombi</i>	USA	Hawaii	Honolulu	Waianae Forest Reserve	21.5009 N, 158.1680 W	UF469195	KT008307	KT008356
<i>Planogyra asteriscus</i>	USA	Maine	Piscataquis	Atkinson Mills	45.1421 N, 69.0312 W	JCN10494	KT008305	KT008364
<i>Pronesopupa boettgeri</i>	USA	Hawaii	Oahu	Kalihi Valley	21.3585 N, 157.8580 W	Hayes	KT008306	KT008354
<i>Ptychalaria tamagonori</i>	Japan	Tokyo	Ogasawara	Chichijima	27.0948 N, 142.2166 E	Chiba	KT008323	KT008348
<i>Pupilla muscorum</i>	Czech Rep.	Moravia		Brno	49.2509 N, 16.5738 E	Horsak	KT008313	KT008361
<i>Pupisoma dioscorticola</i>	USA	Florida	Alachua	Batram-Carr Woods	29.6436 N, 82.3456 W	Slapcinsky	KT008304	KT008357
<i>Sterkia calamitosa</i>	USA	California	San Diego	Sumner Canyon	32.8734 N, 117.2483 W	JCN18169	KT008324	KT008350
<i>Sterkia clementia</i>	USA	California	San Diego	San Clemente Island	32.9953 N, 118.5516 W	JCN19109	KT008325	KT008351
<i>Sterkia hemphilli</i>	USA	California	San Diego	Border Fields State Park	32.5428 N, 117.1061 W	JCN19780	KT008331	KT008352
<i>Strobilops labyrinthica</i>	USA	Michigan	Mackinac	Brevort Lake	45.9829 N, 84.8618 W	JCN17151	KT008309	KT008362
<i>Truncatellina callicratis</i>	France	Normandy	Calvados	Pont, N. of Falaise	48.9774 N, 0.0902 W	BC00002	KT008302	KT008358
<i>Truncatellina cylindrica</i>	Ukraine	Crimea		Rozovyi	44.6920 N, 34.3190 E	Cameron	KT008303	KT008359
<i>Vallonia costata</i>	USA	New Mexico	Bernalillo	Albuquerque	35.0727 N, 106.6160 W	JCN	KT008312	
<i>Vertigo alabamensis</i>	USA	Florida	Leon	Wolf Trap Bay	30.3680 N, 84.5700 W	JCN12323	KF214500	KT008339
<i>Vertigo antivertigo</i>	UK	Wales	Anglesey	Waun Eraud Fen	53.3008 N, 4.2411 W	JCN	KT008316	KT008338
<i>Vertigo clappi</i>	USA	Tennessee	Carter	Lynn Mountain	36.3545 N, 82.1535 W	UF299578	KT008321	KT008337
<i>Vertigo cristata</i>	USA	Wisconsin	Oneida	Sugar Camp Bog	45.8499 N, 89.2958 W	JCN12213	GQ921543	KT008344
<i>Vertigo gouldii</i>	USA	West Virginia	Lincoln	Big Ugly WMA	38.0859 N, 82.0009 W	Doursen	KT008330	KT008333
<i>Vertigo cf. okinerabuensis</i>	Japan	Iwate	Ichinoseki	Sarusawa	38.9869 N, 141.2550 E	Chiba	JN941044	KT008341
<i>Vertigo malleata</i>	USA	North Carolina	Pender	Holly Shelter Game Land	34.5492 N, 77.7817 W	UF449308	KT008318	KT008345
<i>Vertigo meramecensis</i>	USA	Kentucky	Bullitt	Cave Hollow	37.9379 N, 85.6334 W	JCN	KT008329	KT008335
<i>Vertigo milium</i>	USA	Iowa	Buchanan	Rowley North fen	42.3764 N, 91.8507 W	JCN5747	KT008328	KT008346
<i>Vertigo moulinsiana</i>	Netherlands	South Holland	Kaag en Braassem	Woubrugge, Widje Aa	52.1710 N, 4.6119 E	JCN	KT008326	KT008334
<i>Vertigo pseudosubstriata</i>	Russia	Altai Republic		Seminski Pass S	50.9855 N, 85.6817 E	JCN	KT008317	KT008343
<i>Vertigo pusilla</i>	Czech Rep.	Moravia		Podyji National Park	48.8586 N, 15.8960 E	Horsak	KF214496	KT008342
<i>Vertigo pygmaea</i>	Czech Rep.	Moravia		Kotrlé Fen	49.3779 N, 18.0236 E	JCN	KT008322	KT008340
<i>Vertigo rugosula</i>	USA	Arkansas	Jefferson	Lock & Dam #5	34.4040 N, 92.1020 W	UF409059	KT008319	KT008332
<i>Vertilla angustior</i>	Czech Rep.	Moravia		Pozdechov Fen	49.2339 N, 17.9864 E	JCN	KT008320	KT008336
<i>Zoogenetes harpa</i>	Canada	Quebec	Manicougan	Pointe-des-Montes	49.3256 N, 67.3700 W	JCN13609	KT008311	

Abbreviations: BC, Coles collection in National Museum of Wales, of which the accession numbers are to be preceded by 'NMW.Z.2015.009'; JCN, Nekola collection in University of New Mexico; UF, material in Florida Museum of Natural History, Gainesville.

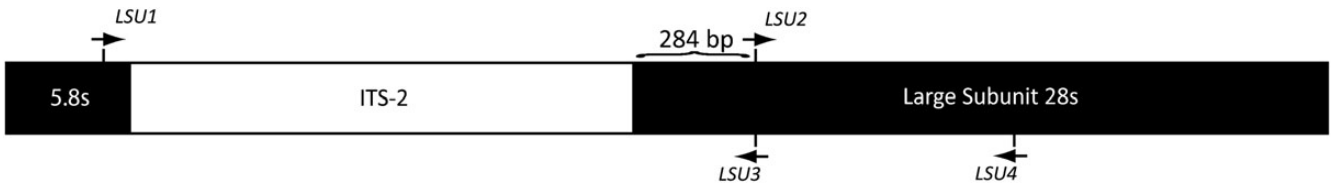


Figure 1. Map of the rRNA gene cluster with primer locations for the analysed segment of 28S in relation to the 5.8S and ITS-2 regions.

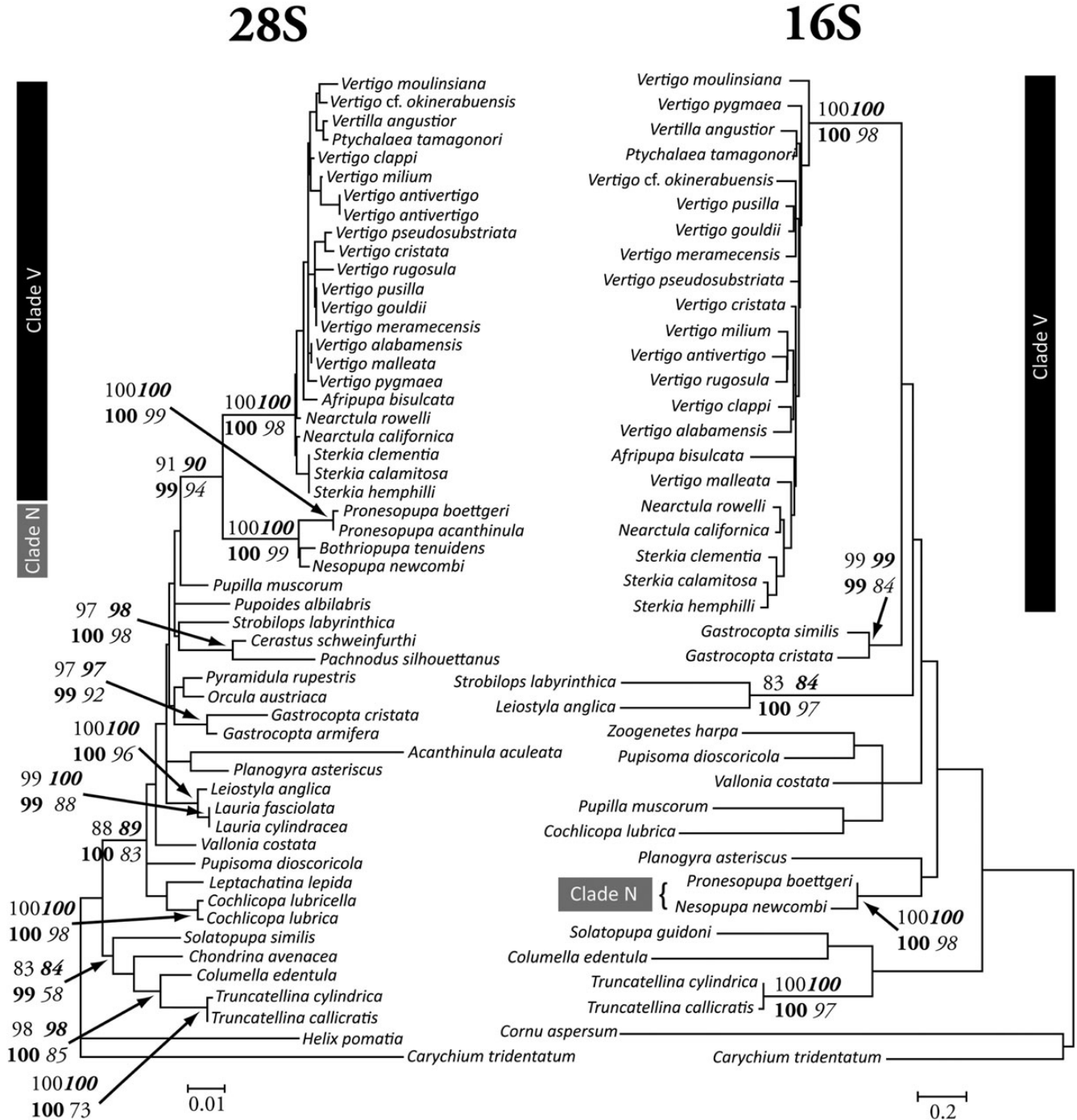


Figure 2. Maximum-likelihood phylogenetic tree reconstruction based on nuclear 28S (left) and mitochondrial 16S (right) data. Nodes with strong to moderate support (≥ 70) across all four phylogenetic reconstruction methods have been labelled to the left of that node by four support values: upper left (normal font) is for NJ; upper right (**bold italic font**) is for MP; lower left (**bold font**) is for Bayesian; lower right (*italic font*) is for ML.

and transversions with pairwise gap deletion. MP analysis used the close-neighbour interchange search option with the random addition of 10 replicate trees. ML analysis used all sites and was based on the Tamura-Nei substitution model, a five-category gamma distribution for substitution rates and the nearest neighbour interchange ML heuristic method. In all cases support values were estimated from 1,000 bootstrap replicates. Additionally, Bayesian trees were generated using MrBayes v. 3.1 (Huelsenbeck & Ronquist, 2001), with a generalised time reversible substitution model assuming gamma-shaped rate variation over 1,000,000 generations and a sampling frequency of once each 1,000 generations.

Analysis of these data (Fig. 2) demonstrates poorer resolution using the mitochondrial 16S as compared with the nuclear 28S data, presumably due to higher base-pair saturation rates. Therefore, we used the 28S data to deduce phylogenetic relationships and the 16S data for quasi-independent corroboration.

The 28S data show a highly-supported clade composed of two highly-supported sister clades, together including most of the putative Vertigininae (Clade V) and Nesopupinae (Clade N) included in this study. This ‘vertiginid’ clade in general corresponds with the Vertiginidae as outlined by Bouchet & Rocroi (2005) and Pokryszko *et al.* (2009), with the marked exceptions that: (1) *Gastrocopta* is excluded as it is neither closely related to *Vertigo* nor to the chondrinids, but rather represents a distinct branch within the Orthurethra, and (2) both *Columella* and *Truncatellina* are excluded as both are members of a moderately supported clade that includes *Chondrina*. Wade *et al.* (2001, 2006) have shown that the chondrinids are actually sister to the Orthurethra. The analysis of 16S data in general corroborates these findings, albeit without resolving the sister status of clades V and N.

The 28S and 16S trees both demonstrate that Clade N consists only of tropical species historically assigned to the Nesopupinae. These are known to differ anatomically from the Vertigininae by possessing a penial appendix and a forked retractor muscle (Pilsbry, 1919). Clade V includes not only all analysed *Vertigo*, but also the putative genera *Nearctula* and *Vertilla* and members of some genera historically assigned to the Nesopupinae (*Afripupa*, *Ptychalaia* and *Sterkia*). Pilsbry (1919) lacked anatomical data for any species within these latter three genera and placed them outside of *Vertigo* solely on biogeographical (tropical/subtropical range) and conchological grounds (pustulate shell sculpture, and/or strength and location of angular lamella). Previous analyses have shown that these shell features are not reliable for use in supraspecific taxonomy of pupillids (Coles & Nekola, 2007; Nekola *et al.*, 2009; Nekola, Coles & Horsák, 2015).

It should also be noted that for both the 28S and 16S trees, monophyly of *Vertigo* is only preserved when the taxonomic concept of the genus is expanded to include all members of Clade V. Because support levels for this clade are similar to other apparent genus-level clades (including *Cochlicopa*, *Gastrocopta*, *Lauria* and *Truncatellina*), our data suggest that it would be most parsimonious to assign all species within Clade V to the genus *Vertigo*. Our more limited sampling and representation of type species in the narrowly-constrained Nesopupinae (Clade N) makes it impossible for us to say how many generic-level entities might be supported in that group. However it is interesting to note that the genetic divergence between *Bothriopupa* and *Nesopupa* is well within the range found within *Vertigo*. It thus remains an open question how many genera within the Nesopupinae will ultimately be supported by DNA sequence data.

These data do not support subgeneric classification within *Vertigo*, given that no nodes within Clade V possess support values >70 in either 28S or 16S analyses. However, the weakly supported monophyly of *Nearctula*/*Sterkia* suggests that useful subgeneric groupings may in fact exist. Consideration of these issues must await analysis of other amplicons that are more

rapidly evolving (therefore with more taxonomically useful sites) than 28S, but more slowly evolving (thus with lower base-pair saturation rates) than 16S.

If retained, the family Vertiginidae represents a highly supported clade possessing only two branches: *Vertigo* on one hand and a subset of nesopupids on the other. But how useful is recognition of so small a family? We agree with Puillandre *et al.* (2015) that such decisions are ultimately matters of taxonomic opinion that cannot be subjected to empirical criteria. In this we freely admit to erring on the side of conservatism, as we do not see the utility of erecting a large number of high-level taxonomic groups that constitute only single branches or simple two-branch entities when a more inclusive division containing more taxa is possible by simply stepping back one level in the tree.

Even though outside our primary focus, these analyses also hint that other traditional supraspecific concepts within the Orthurethra may be in need of revision. In particular, the Valloniidae may be polyphyletic with both *Pupisoma* and *Acanthinula*/*Planogyra* each representing their own unique branches within the Orthurethra. The 16S data also suggest that *Zoogoneles* may be more closely related to *Pupisoma*. Pilsbry (1927: vii) appears to have been prescient when he stated “The division of the Orthurethra into families seems to be largely a matter of expediency.” These empirical data validate this statement and strongly suggest that formal reconsideration of supraspecific concepts across the entire infraorder are warranted, based upon DNA sequence data.

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