

Nymoen AR, Kongsrud JA, Willassen E, Bakken T. When standard DNA-barcodes do not work for species identification: Intermixed mitochondrial haplotypes in the Jaera albifrons complex (Crustacea: Isopoda). Marine Biodiversity. Contact: torkild.bakken@ntnu.no
Table 1a

	Results From Tajima's Neutrality Test						sign	interpretation
	m	S	Ps	Θ	π	D		
all ingroup	77	26	0.045217	0.009201	0.006595	-0.87585	P > 0.10	neutral
sarsi	5	1	0.001739	0.000835	0.000696	-0.816497	P > 0.10	neutral
ischiosetosa	18	11	0.01913	0.005562	0.005001	-0.36784	P > 0.10	neutral
forsmani	7	10	0.017391	0.007098	0.006957	-0.107316	P > 0.10	neutral
albifrons	26	16	0.027826	0.007292	0.00686	-0.207195	P > 0.10	neutral
praehirsuta	19	11	0.01913	0.005473	0.005899	0.279421	P > 0.10	neutral

Table. Results from Tajima's Neutrality Test

NOTE. This analysis involved 70 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 575 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]
Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic (see chapter 12 in ref. [3] for details).

- 1. Tajima F. (1989). Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 123:585-595.
- 2. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution <https://doi.org/10.1093/molbev/msab120>.
- 3. Nei M. and Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Table 1b McDonald-Kreitmann test

all ingroup Synonymous Substitutions: Fixed differences between species: 97 Polymorphic sites: 26 Nonsynonymous Substitutions: Fixed differences between species: 27 Polymorphic sites: 1	praehirsuta Synonymous Substitutions: Fixed differences between species: 103 Polymorphic sites: 11 Nonsynonymous Substitutions: Fixed differences between species: 30 Polymorphic sites: 1	albifrons Synonymous Substitutions: Fixed differences between species: 102 Polymorphic sites: 15 Nonsynonymous Substitutions: Fixed differences between species: 27 Polymorphic sites: 1	forssmani Synonymous Substitutions: Fixed differences between species: 103 Polymorphic sites: 10 Nonsynonymous Substitutions: Fixed differences between species: 30 Polymorphic sites: 1	ischiosetosa Synonymous Substitutions: Fixed differences between species: 104 Polymorphic sites: 9 Nonsynonymous Substitutions: Fixed differences between species: 30 Polymorphic sites: 1	all ingroup Synonymous Substitutions: Fixed differences between species: 97 Polymorphic sites: 26 Nonsynonymous Substitutions: Fixed differences between species: 27 Polymorphic sites: 1
Neutrality Index, Ni: 0.138 Alpha value: 0.862	Neutrality Index, Ni: 0.312 Alpha value: 0.688	Neutrality Index, Ni: 0.252 Alpha value: 0.748	Neutrality Index, Ni: 0.343 Alpha value: 0.657	Neutrality Index, Ni: 0.385 Alpha value: 0.615	Neutrality Index, Ni: 0.138 Alpha value: 0.862
Fisher's exact test. P-value (two tailed): 0.028653*	Fisher's exact test. P-value (two tailed): 0.308663 (not significant)	Fisher's exact test. P-value (two tailed): 0.199721 (not significant)	Fisher's exact test. P-value (two tailed): 0.457034 (not significant)	Fisher's exact test. P-value (two tailed): 0.463757 (not significant)	Fisher's exact test. P-value (two tailed): 0.028653*
G test. G value: 6.303 P-value: 0.01205* G value with Williams' correction: 6.084 P-value: 0.01364* G value with Yates' correction: 4.562 P-value: 0.03270*	G test. G value: 1.601 P-value: 0.20570 (not significant) G value with Williams' correction: 1.498 P-value: 0.22103 (not significant) G value with Yates' correction: 0.687 P-value: 0.40706 (not significant)	G test. G value: 2.457 P-value: 0.11698 (not significant) G value with Williams' correction: 2.324 P-value: 0.12736 (not significant) G value with Yates' correction: 1.323 P-value: 0.24997 (not significant)	G test. G value: 1.301 P-value: 0.25412 (not significant) G value with Williams' correction: 1.210 P-value: 0.27137 (not significant) G value with Yates' correction: 0.484 P-value: 0.48661 (not significant)	G test. G value: 0.992 P-value: 0.31930 (not significant) G value with Williams' correction: 0.916 P-value: 0.33845 (not significant) G value with Yates' correction: 0.293 P-value: 0.58810 (not significant)	G test. G value: 6.303 P-value: 0.01205* G value with Williams' correction: 6.084 P-value: 0.01364* G value with Yates' correction: 4.562 P-value: 0.03270*
* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001	* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001	* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001	* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001	* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001	* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001

a neutrality index lower than 1 (i.e. Ni < 1) indicates an excess of nonsilent divergence, which occurs when positive selection is at work in the population
Alpha represents the proportion of substitutions driven by positive selection

Both the two-tailed Fisher's exact test, and G-test of independence are computed to determine whether the deviations on the ratio of replacement to synonymous (fixed substitutions between species vs. polymorphisms within species) are or not significant. DnaSP obtains the probability associated with the G value (with 1 degree of freedom) by the trapezoidal method of numerical integration.

Pn/Ps/Dn/Ds 0.138

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 Submitted to Marine Biodiversity. Contact: torkild.bakken@ntnu.no
 Table 1c Codon-based Z-Test

	prob	stat
sarsi	1,000	-1,027
ishiosetosæ	0,002	2,977
forsmani	0,002	2,930
albifrons	0,000	4,088
præhirsutæ	0,003	2,746

Table. Codon-based Z-Test of Purifying Selection for analysis averaging over all sequence pairs within each group. The probability of rejecting the null hypothesis of strict-neutrality ($dN = dS$) in favor of the alternative hypothesis ($dN < dS$) (in the Prob column) is shown. Values of P less than 0.05 are considered significant at the 5% level and are highlighted. The test statistic ($dS - dN$) is shown in the Statcolumn. dS and dN are the numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the bootstrap method (200 replicates). Analyses were conducted using the Nei-Gojobori method [1]. This analysis involved 75 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 191 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]

1. Nei M. and Gojobori T. (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Molecular Biology and Evolution 3:418-426.
2. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution <https://doi.org/10.1093/molbev/msab120>.

Supplementary Tables 2 and 3

Nymoen AR, Kongsrud JA, Willassen E, Bakken T. When standard DNA-barcodes do not work for species identification: Intermixed mitochondrial haplotypes in the *Jaera albifrons* complex (Crustacea: Isopoda). Marine Biodiversity. Contact: torkild.bakken@ntnu.no

Table S2 Results from ASAP analysis of CO1 sequences from *Jaera albifrons* group and outgroup species *Jaera sarsi*. The best ASAP score indicates five species, while the partition with two species has the highest probability.

Nb of subsets	asap-score	P-val (rank)	W (rank)	Threshold dist.
5	3.00	6.29e-01 (4)	1.06e-05 (2)	0.004340
9	3.50	5.95e-01 (3)	7.30e-06 (4)	0.001745
* 3	3.50	9.02e-01 (6)	1.22e-05 (1)	0.006076
2	4.50	1.00e-05 (1)	-4.21e-05 (8)	0.121528
27	4.50	4.50e-03 (2)	4.37e-06 (7)	0.000868
8	5.00	7.92e-01 (5)	6.50e-06 (5)	0.002612
10	5.00	9.52e-01 (7)	7.30e-06 (3)	0.001738

Table S3. Results from ASAP analysis of 28S sequences from *Jaera albifrons* group and outgroup species *Jaera caspica*, *Jaera hopeana*, and *Jaera massiliensis*. The best ASAP score indicates three species, which implies lumping of *J. caspica* and *J. hopeana*, and that the *J. albifrons* group is one (panmictic) species.

Nb of subsets	asap-score	P-val (rank)	W (rank)	Threshold dist.
3	1.50	1.00e-05 (1)	1.30e-02 (2)	0.230091
3	3.00	1.74e-01 (3)	1.76e-03 (3)	0.285441
4	3.00	1.00e+00 (5)	2.85e-02 (1)	0.104212
6	4.00	1.28e-01 (2)	1.72e-05 (6)	0.000502
5	4.00	8.68e-01 (4)	6.77e-05 (4)	0.001006