*J. Mar. Biol. Ass. U.K.* (1999), **79**, 899–906
Printed in the United Kingdom

# Allozymic analysis of some Mediterranean Veneridae (Mollusca: Bivalvia): preliminary notes on taxonomy and systematics of the family

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An allozyme analysis on three species of Mediterranean Veneridae (Mollusca: Bivalvia): *Callista chione* (subfamily Pitarinae), *Venus verrucosa* (Venerinae) and *Chamelea gallina* (Chioninae) is presented. The results were compared with the previously obtained allozyme data on *Tapes philippinarum*, *T. decussatus*, *Venerupis aurea* and *Paphia undulata* (all pertaining to the subfamily Tapetinae). The newly analysed species showed remarkable departures from the Hardy–Weinberg proportions, as commonly observed in several populations of Bivalvia. The present analysis revealed a high level of relationship between *C. gallina* and *Venus verrucosa* and suggested that the genus *Tapes*, as presently defined, should be regarded as an unnatural taxon. The allozyme results are consistent with mitochondrial 16s ribosomal DNA and satellite DNAs analyses on the same species, with the only exception of *Callista chione* which seems to be basal to (UPGMA method) or even included (Neighbor-Joining method) in the Tapetinae cluster.

## INTRODUCTION

The venerids are a large family of infaunal, shallow-burrowing, suspension filter feeders, which are mainly distributed throughout the temperate-tropical seas. They comprise as many as 500 species, split into 50 extant and 55 extinct genera. At variance with the majority of the bivalve families, the evolutionary history of the Veneridae is quite recent: early fossil records date back to the Cenozoic and suggest a wide species radiation, possibly of polyphyletic origin (Keen, 1969).

The family has been split into 12 subfamilies, mainly on the basis of shell sculpture, pallial line and dentition features. However, this splitting does not seem to adequately reflect real phylogenetic relationships among taxa and it is adopted mainly for convenience in arrangements of venerids (Keen, 1969). Morphological characters of the shell have been widely utilized also for the taxonomy at the genus level, although shell features of Veneridae are highly homogeneous and deeply influenced by environmental adaptations. Actually, several cases of convergence in conchological characters in distantly related species and, conversely, of extensive conchological diversification among closely related species have been observed (Harte, 1992). Therefore, the morphological taxonomy of the Veneridae needs to be carefully checked taking into account results obtained through new approaches such as the biochemical and the molecular ones. Allozyme electrophoretic analyses (Borsa & Thiriot-Quiévreux, 1990; Passamonti et al., 1997) and mitochondrial 16S ribosomal DNA characterization (Canapa et al., 1996) have revealed clear inconsistencies of the commonly accepted taxonomic arrangement of some Mediterranean species, both at subfamily and genus level.

In this paper the allozymic characterization of samples of *Callista chione* (Linnaeus, 1758) (subfamily Pitarinae), *Venus verrucosa* Linnaeus, 1758 (Venerinae) and *Chamelea gallina* (Linnaeus, 1758) (Chioninae) are presented. New information is then compared with previously published data on four related taxa of Tapetinae (Passamonti et al., 1997).

## MATERIALS AND METHODS

Allozymatic analyses were carried out on one sample each of *Callista chione* (*Cch*; 28 specimens), *Venus verrucosa* (*Vve*; 14 specimens) and *Chamelea gallina* (*Cga*; 25 specimens) collected in the Venice Lagoon. For appropriate comparisons samples of *Tapes philippinarum* (*Tph*; 94 specimens, from Venice Lagoon, Scardovari Lagoon, Cesenatico and Rimini), *T. decussatus* (*Tde*; 72 specimens, from Venice Lagoon, Rimini, S. Benedetto del Tronto and Sfax), *Venerupis aurea* (*Vau*; 64 specimens, from Rimini, Porto S. Giorgio and Giulianova) and *Paphia undulata* (*Pun*; 20 specimens, from Thailand), analysed in Passamonti et al. (1997), were taken into account. All sampling localities are shown in Figure 1, with the only exception of *P. undulata* from Thailand.

Sample preparations and electrophoretic conditions were according Passamonti et al. (1997). Utilized tissues, run substrates, staining procedures, assayed loci and enzymes with their structures are described in Table 1.

At each locus, the commonest allele of *T. philippinarum* was referred to as 100, while other alleles were reported on the basis of their relative mobility, adding or subtracting from the 100 value the corresponding millimetres for faster or slower alleles, respectively.

We assumed a locus to be polymorphic when the frequency of the commonest allele was 0.99 or less and at least another allele frequency was 0.01 or higher. According to this scale, alleles with a frequency lower than 0.01 were not introduced in our calculations.

**Table 1.** *Enzyme names and abbreviations, enzyme codes (E.C.), analysed tissues, run substrates, electrophoretic staining procedure references, number of loci and enzyme structures.*

| Enzyme | E.C. number | Analysed tissues | Run substrates | References for staining procedures | Number of loci | Enzyme structure |
|---|---|---|---|---|---|---|
| Aspartate aminotransferase (AAT) | 2.6.1.01 | mu | ca | Meera Khan et al., 1982 | 2 | D |
| Adenilate chinase (ADK) | 2.7.4.03 | he | sg | Van Someren et al., 1974 | 1 | M |
| Aldolase (ALD) | 4.1.2.13 | mu | ca | Benson & Smith, 1989 | 2 | M |
| Fumarate hydratase (FH) | 4.2.1.2 | mu | sg | Meera Khan et al., 1982 | 1 | M |
| Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) | 1.2.1.12 | mu | ca | Ayala et al., 1972 | 1 | D |
| α Glycerophosphate dehydrogenase (α GPDH) | 1.1.1.08 | mu | ca | Ayala et al., 1972 | 1 | M |
| Glucosephosphate isomerase (GPI) | 5.3.1.09 | mu | ca | Van Someren et al., 1974 | 1 | D |
| Isocitrate dehydrogenase (IDH) | 1.1.1.42 | he | sg | Meera Khan et al., 1982 | 2 | D |
| Malate dehydrogenase (MDH) | 1.1.1.37 | mu | ca | Meera Khan et al., 1982 | 2 | D |
| Sorbitol dehydrogenase (SDH) | 1.1.1.14 | he | sg | Benson & Smith, 1989 | 1 | M |
| Phosphoglucomutase (PGM) | 2.7.5.01 | mu | ca | Meera Khan et al., 1982 | 1 | M |
| 6-Phosphogluconate dehydrogenase (6PGDH) | 1.1.1.44 | mu | ca | Meera Khan, 1971 | 1 | D |

ca, cellulose acetate membrane; D, dimeric; he, hepatopancreas; M, monomeric; mu, muscle; sg, starch gel.

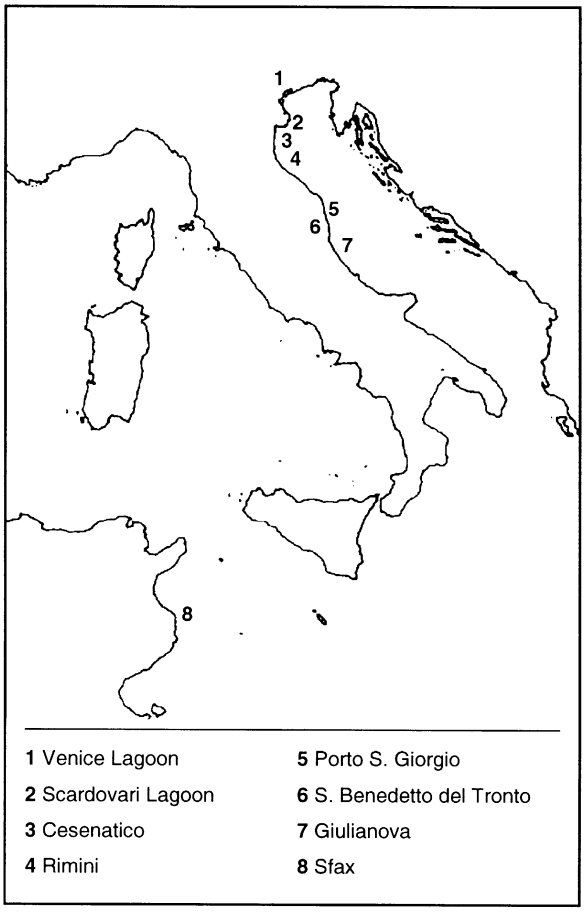


**Figure 1.** Sampling localities in the Mediterranean basin. The newly analysed Veneridae species (*Venus verrucosa*, *Chamelea gallina* and *Callista chione*) were collected in the Venice Lagoon.

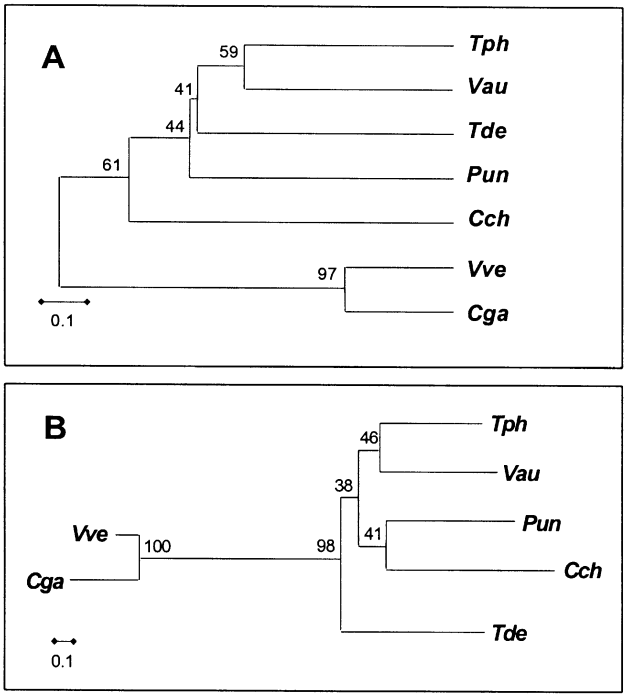


**Figure 2.** Dendrograms showing the phylogenetic relationships among analysed Veneridae species: (A) UPGMA; (B) Neighbor-Joining. Bootstrap values are reported at branches. Species notation as in Appendix 1.

In *Venus verrucosa*, *C. gallina* and *Callista chione*, Hardy–Weinberg disequilibria were tested with the exact test (Ex; Elston & Forthofer, 1977) and the *f* estimator (Weir & Cockerham, 1984), using Genepop software (Raymond & Rousset, 1995). Wright's $F_{is}$ (Weir & Cockerham, 1984)



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**Table 2.** *Wright's* $F_{is}$ *for the three newly analysed species. Significance levels as in Appendix 1.*

| *Loci* | $F_{is}$ *Vve* | *Cga* | *Cch* |
|---|---|---|---|
| *αGpdh* | 0.431 | — | — |
| *Sdh* | 1.000 | 0.245 d | 0.478 |
| *Mdh*-1 | — | — | 0.477 |
| *Mdh*-2 | — | −0.042 | 0.410 c |
| *Idh*-1 | 0.000 | — | — |
| *Idh*-2 | −0.040 | 0.633 c | 0.664 c |
| *6Pgdh* | 0.307 | −0.055 | 0.224 |
| *G3pdh* | 0.224 | 0.610 a | 0.655 b |
| *Aat*-1 | 0.762 d | — | — |
| *Aat*-2 | 0.721 a | 0.380 a | 0.000 |
| *Adk* | — | −0.022 | 1.000 a |
| *Pgm* | 0.281 d | 0.126 | 0.113 |
| *Ald*-2 | 1.000 d | −0.001 | — |
| *Fh* | — | 1.000 a | 1.000 d |
| *Gpi* | 0.190 | 0.083 | 0.218 |
| *tot* | 0.451 a | 0.244 a | 0.507 a |

**Table 3.** *Nei's genetic distances for the seven analysed Veneridae species. Species notations as in Appendix 1.*

| | *Tph* | *Tde* | *Vau* | *Pun* | *Vve* | *Cga* | *Cch* |
|---|---|---|---|---|---|---|---|
| *Tph* | — | | | | | | |
| *Tde* | 1.09 | — | | | | | |
| *Vau* | 0.90 | 1.16 | — | | | | |
| *Pun* | 1.32 | 1.20 | 1.07 | — | | | |
| *Vve* | 1.48 | 1.41 | 1.88 | 1.64 | — | | |
| *Cga* | 1.78 | 1.86 | 1.70 | 2.02 | 0.44 | — | |
| *Cch* | 1.31 | 1.61 | 1.48 | 1.33 | 1.72 | 1.96 | — |

was calculated per polymorphic locus; averages of $F_{is}$ over all loci and the corresponding standard deviations were obtained using the Jackknife estimator (Weir, 1990). Their significance was evaluated through the permutation procedure (10,000 steps). Values of Wright's $F_{is}$ were obtained using FSTAT software (Goudet, 1995). No calculation on genotypic structure was performed on samples exhaustively analysed in Passamonti et al. (1997).

Genetic distance matrices were estimated according to Nei (1972). Dendrograms were obtained following the UPGMA (Sneath & Sokal, 1973) and the Neighbor-joining (Saitou & Nei, 1987) methods. In each dendrogram confidence limits of the clusters were evaluated through bootstrap analysis (Felsenstein, 1985). Values of genetic distance, dendrogram and bootstrap were obtained by the PHYLIP computing programs (3.5 ©Macintosh executable version, copyright 1986–1993 by Joseph Felsenstein).

## RESULTS

The newly analysed samples gave readable patterns at all assayed enzyme systems for a total number of 16 enzyme loci. For the *Cch* sample (*Callista chione*) no enzymatic activity was observed at the *Idh*-1 locus (Appendix 1). All loci were shown to be highly polymorphic, with the only exception of the *Ald*-1 locus. When observed vs expected heterozygosity values differed significantly, the Exact test (Ex) and *f*-values showed that it was invariably due to heterozygote deficiency. In particular, for *Adk* of *C. chione*, *Ald*-2 of *Venus verrucosa* and *Fh* of *Chamelea gallina* and *Callista chione*, not a single heterozygote was scored ($f=1.000$) (Appendix 1). Also $F_{is}$ values supported the observed Hardy–Weinberg disequilibria in those samples: intrapopulation genotypic heterogeneity was commonly observed at most loci and significant shifts from panmictic proportions were always due to heterozygote deficiency (Table 2).

When all taxa were considered and genetic distances calculated, a wide range of genetic differentiation emerged, with D values ranging from 0.44 to 2.02. In detail, the lowest genetic distance value among species was obtained between *Chamelea gallina* and *V. verrucosa* (D=0.44), followed by the one between *Tapes philippinarum* and *Venerupis aurea* (D=0.90). As expected from the established taxonomy, Tapetinae species showed lower levels of pair-wise differentiation between them (D=0.90–1.32) than when compared to species belonging to different subfamilies (D=1.31–2.02). From these comparisons, *Callista chione* (Pitarinae) appeared to be more related to the Tapetinae group (D=1.31–1.61) than to *Venus verrucosa* (Venerinae) and *Chamelea gallina* (Chioninae) (D=1.72 and 1.96, respectively) (Table 3). It should be mentioned that for *T. philippinarum*, *T. decussatus* and *Venerupis aurea* intraspecific distances ranged from 0.017 to 0.242 (Passamonti et al., 1997).

As anticipated by distance values, the UPGMA clustering evidenced a relatively low level of differentiation between *Venus verrucosa* and *C. gallina* (Figure 2A) and joined the two species with a very good bootstrap value (97%). For the remaining taxa, the UPGMA appeared to support the above-observed high, homogeneous level of differentiation. The Neighbor-Joining tree (Figure 2B) suggests a relatively higher level of relatedness between *V. verrucosa* and *C. gallina* (100% bootstrap value), and pools all other species in the same cluster with a very good bootstrap value (98%). However, in UPGMA the branching pattern of the taxa is different from Neighbor-Joining trees.

## DISCUSSION

Departures from Hardy–Weinberg equilibrium appear to be a common feature of bivalve genetic structure, since it is known to occur also in Pectinidae, Mytilidae, Ostreidae and Veneridae (Zouros & Foltz, 1984; Borsa et al., 1991). A wide range of different hypotheses has been proposed to explain the commonly observed Hardy–Weinberg disequilibria, including the presence of lethal 'null' alleles, the selection against heterozygotes during the larval stage or a 'temporal' Wahlund effect (Borsa et al., 1991 and references therein). The latter hypothesis should be related to year cohorts' heterogeneities in allelic frequencies, brought about by differences in selection from year to year. Such differences have been observed in French populations of *Tapes decussatus* (Borsa et al., 1991); however, whether the same holds true for additional Veneridae species has not been tested yet. The data reported here on *Callista chione*, *Venus verrucosa*



https://doi.org/10.1017/S0025315498001064 Published online by Cambridge University Press

and *Chamelea gallina* show heterozygote deficiencies at several loci, as observed for other venerid species (Passamonti et al., 1997), but it is not suitable for specifically backing any of the above mentioned hypotheses.

Present analysis provides additional evidence of the inadequacy of the morphological venerid taxonomy. As a matter of fact, the ascertained level of genetic affinity between *C. gallina* and *V. verrucosa* was quite unexpected since the two taxa are actually ascribed to different subfamilies (Chioninae and Venerinae, respectively). Yet, 16S rDNA analysis also clearly indicates a relatively close affinity between them (Canapa et al., 1996); therefore two independent approaches suggest that the two species are genetically closer than generally believed (Keen, 1969). It should also be recalled that on morphological grounds Fischer-Piette (1975) suggested Chioninae and Venerinae subfamilies should not be considered as distinct. Investigations on some more taxa are now planned to test the polyphyly of the above mentioned subfamilies.

The high but quite homogeneous level of genetic divergence among the other species becomes a poor cluster resolution. Nonetheless, the genus *Tapes*, as presently defined, should be regarded as an unnatural taxon: although the cluster topology is not significantly supported (95% criterion, Felsenstein, 1985), the genotypic divergence of the two analysed *Tapes* species is of the same magnitude as that observed in intergeneric comparisons of Tapetinae. The high genetic divergence between *T. decussatus* and *T. philippinarum* is also supported by both mitochondrial 16S rDNA (Canapa et al., 1996) and Satellite DNAs studies (Passamonti et al., 1998).

On the other hand, the allozyme and nucleotide sequence analyses indicate different affinities for *Callista chione*. In 16S rDNA analysis, the taxon clusters with *Pitar rudis* (another Pitarinae species) and no close relationship to Tapetinae taxa could be established for it (Canapa et al., 1996). On the contrary, gene-enzyme analysis points to an affinity of *C. chione* with Tapetinae taxa. Further investigations on Pitarinae species therefore appear relevant to better address the systematic position of *C. chione* and the phylogenetic consistence of the Pitarinae subfamily as a whole.

In conclusion, biochemical and molecular evidence stress the need for a deep revision of the Veneridae systematics, by applying biochemical and molecular approaches to a larger number of species. All this should help in a safer tracing of the natural evolutionary tree of the Veneridae.

This work has been supported by Italian Ministero dell' Università e della Ricerca Scientifica e Tecnologica and Consiglio Nazionale delle Ricerche grants.

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*Submitted 4 August 1998. Accepted 7 December 1998.*

**Appendix 1.** *Allelic frequencies of seven Veneridae species at 16 enzyme loci.*

| Loci | Alleles | Species *Tph* | *Tde* | *Vau* | *Pun* | *Vve*# | *Cga*# | *Cch*# |
|---|---|---|---|---|---|---|---|---|
| **Aat*-1 | 90 | — | — | — | — | 0.773 | 1.000 | — |
| | 94 | — | — | — | — | — | — | 1.000 |
| | 100 | 0.838 | — | — | — | — | — | — |
| | 106 | 0.134 | — | — | — | 0.227 | — | — |
| | 110 | — | — | 1.000 | — | — | — | — |
| | 111 | 0.028 | — | — | — | — | — | — |
| | 112 | — | 1.000 | — | 0.125 | — | — | — |
| | 119 | — | — | — | 0.875 | — | — | — |
| N | | 71 | 44 | 44 | 8 | 11 | 8 | 16 |
| Ex | | | | | | d | | |
| *f* | | | | | | 0.762 | | |
| **Aat*-2 | 95 | — | 1.000 | — | — | — | — | — |
| | 100 | 0.860 | — | — | — | — | — | — |
| | 105 | — | — | — | — | — | 0.043 | — |
| | 106 | 0.140 | — | — | — | — | — | — |
| | 108 | — | — | — | 0.028 | — | 0.130 | 0.980 |
| | 111 | — | — | 0.066 | 0.972 | 0.143 | — | — |
| | 112 | — | — | — | — | — | 0.065 | — |
| | 114 | — | — | — | — | 0.250 | — | — |
| | 115 | — | — | — | — | — | 0.109 | 0.020 |
| | 117 | — | — | 0.226 | — | 0.071 | 0.065 | — |
| | 119 | — | — | — | — | 0.036 | 0.218 | — |
| | 121 | — | — | 0.010 | — | — | 0.022 | — |
| | 123 | — | — | 0.377 | — | — | 0.065 | — |
| | 125 | — | — | — | — | 0.429 | 0.218 | — |
| | 126 | — | – | 0.047 | — | — | — | — |
| | 128 | — | — | 0.028 | — | — | — | — |
| | 131 | — | — | 0.094 | — | 0.071 | 0.065 | — |
| | 133 | — | — | 0.123 | — | — | — | — |
| | 139 | — | — | 0.019 | — | — | — | — |
| | 141 | — | — | 0.010 | — | — | — | — |
| N | | 82 | 62 | 53 | 18 | 14 | 23 | 25 |
| Ex | | | | | | a | a | |
| *f* | | | | | | 0.721 | 0.360 | |
| **Adk* | 88 | — | — | — | — | 1.000 | 0.962 | — |
| | 91 | — | — | — | — | — | 0.038 | — |
| | 100 | 1.000 | 1.000 | 1.000 | 1.000 | — | — | 0.760 |
| | 102 | — | — | — | — | — | — | 0.200 |
| | 104 | — | — | — | — | — | — | 0.040 |
| N | | 60 | 27 | 24 | 15 | 12 | 26 | 25 |
| Ex | | | | | | | | a |
| *f* | | | | | | | | 1.000 |
| **Ald*-1 | 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| N | | 16 | 16 | 16 | 12 | 14 | 24 | 27 |
| **Ald*-2 | 92 | — | — | — | — | — | — | 1.000 |
| | 96 | — | 0.344 | — | 0.625 | — | — | — |
| | 98 | — | 0.063 | 0.624 | 0.292 | — | — | — |
| | 100 | 1.000 | 0.593 | — | 0.083 | 0.714 | — | — |
| | 102 | — | — | 0.063 | — | 0.286 | 0.925 | — |
| | 105 | — | — | 0.313 | — | — | 0.075 | — |
| N | | 16 | 16 | 16 | 12 | 7 | 20 | 22 |
| Ex | | | | | | d | | |
| *f* | | | | | | 1.000 | | |
| **Fh* | 90 | — | — | — | — | — | 0.042 | — |
| | 92 | — | — | — | — | — | — | 0.957 |
| | 94 | — | 1.000 | — | — | — | 0.042 | — |
| | 96 | — | — | — | — | 1.000 | 0.916 | 0.043 |
| | 98 | — | — | 1.000 | — | — | — | — |

*Continued.*



https://doi.org/10.1017/S0025315498001064 Published online by Cambridge University Press

**Appendix 1.** *Continued.*

| Loci | Alleles | Species *Tph* | *Tde* | *Vau* | *Pun* | *Vve*# | *Cga*# | *Cch*# |
|---|---|---|---|---|---|---|---|---|
| | 100 | 1.000 | — | — | — | — | — | — |
| | 102 | — | — | — | 1.000 | — | — | — |
| | 108 | — | — | — | — | — | — | — |
| N | | 15 | 14 | 15 | 16 | 13 | 24 | 23 |
| Ex | | | | | | | b | d |
| *f* | | | | | | | 1.000 | 1.000 |
| *G3pdh* | 97 | — | — | — | nd | — | 0.130 | — |
| | 100 | 0.982 | — | — | nd | — | 0.327 | — |
| | 102 | — | 0.323 | — | nd | — | 0.087 | 0.639 |
| | 104 | — | — | — | nd | — | 0.174 | — |
| | 106 | — | — | 0.039 | nd | — | — | — |
| | 107 | 0.018 | — | — | nd | 0.375 | 0.174 | 0.361 |
| | 108 | — | — | 0.206 | nd | — | — | — |
| | 109 | — | 0.662 | — | nd | — | — | — |
| | 110 | — | — | — | nd | 0.458 | 0.065 | — |
| | 112 | — | 0.015 | — | nd | — | — | — |
| | 116 | — | — | 0.755 | nd | 0.167 | — | — |
| | 124 | — | — | — | nd | — | 0.043 | — |
| N | | 85 | 65 | 51 | | 12 | 23 | 18 |
| Ex | | | | | | | a | b |
| *f* | | | | | | | 0.634 | 0.655 |
| **α*Gpdh* | 84 | — | 0.011 | — | — | — | — | — |
| | 88 | — | — | — | 0.028 | — | — | — |
| | 93 | — | — | — | 0.389 | 0.292 | — | — |
| | 96 | 0.230 | 0.193 | 0.684 | 0.583 | — | — | — |
| | 98 | 0.115 | 0.796 | — | — | 0.708 | — | — |
| | 100 | 0.434 | — | — | — | — | — | 1.000 |
| | 102 | 0.221 | — | — | — | — | 1.000 | — |
| | 108 | — | — | 0.316 | — | — | — | — |
| N | | 61 | 44 | 19 | 18 | 12 | 10 | 19 |
| **Gpi* | 95 | 0.055 | — | — | — | — | — | — |
| | 97 | 0.110 | — | — | — | — | 0.042 | — |
| | 100 | 0.403 | — | — | — | — | 0.021 | — |
| | 102 | — | 0.022 | 0.108 | — | 0.071 | — | 0.520 |
| | 103 | — | — | — | — | 0.036 | 0.021 | — |
| | 105 | 0.213 | 0.127 | 0.067 | — | 0.179 | 0.042 | — |
| | 107 | 0.043 | 0.134 | 0.067 | — | — | 0.104 | 0.480 |
| | 108 | — | — | — | — | — | 0.042 | — |
| | 110 | 0.073 | 0.523 | 0.225 | — | — | 0.206 | — |
| | 112 | — | 0.112 | 0.092 | 0.306 | 0.250 | — | — |
| | 113 | — | — | 0.058 | — | — | — | — |
| | 115 | 0.036 | 0.082 | 0.108 | — | — | 0.125 | — |
| | 117 | 0.055 | — | 0.042 | 0.694 | 0.357 | 0.104 | — |
| | 119 | — | — | 0.092 | — | 0.107 | 0.167 | — |
| | 121 | — | — | 0.058 | — | — | — | — |
| | 124 | — | — | 0.058 | — | — | 0.021 | — |
| | 126 | — | — | — | — | — | 0.021 | — |
| | 128 | — | — | — | — | — | 0.063 | — |
| | 129 | — | — | 0.025 | — | — | — | — |
| | 133 | — | — | — | — | — | 0.021 | — |
| N | | 82 | 60 | 64 | 18 | 14 | 24 | 25 |
| Ex | | | | | | d | | |
| *f* | | | | | | 0.190 | | |
| *Idh*-1 | 90 | — | 0.327 | — | — | — | — | nd |
| | 91 | — | — | 1.000 | — | — | — | nd |
| | 98 | — | 0.654 | — | — | — | — | nd |
| | 100 | 1.000 | — | — | — | — | — | nd |
| | 101 | — | 0.019 | — | — | — | — | nd |

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**Appendix 1.** *Continued.*

| Loci | Alleles | Species *Tph* | *Tde* | *Vau* | *Pun* | *Vve*# | *Cga*# | *Cch*# |
|---|---|---|---|---|---|---|---|---|
| | 104 | — | — | — | — | 0.964 | 0.926 | nd |
| | 108 | — | — | — | — | 0.036 | 0.037 | nd |
| | 110 | — | — | — | 0.025 | — | — | nd |
| | 112 | — | — | — | — | — | 0.037 | nd |
| | 115 | — | — | — | 0.975 | — | — | nd |
| N | | 51 | 26 | 37 | 20 | 14 | 27 | |
| **Idh*-2 | 86 | — | — | 1.000 | — | — | — | — |
| | 89 | — | — | — | — | — | — | 0.018 |
| | 92 | — | 1.000 | — | — | — | — | 0.018 |
| | 94 | — | — | — | 1.000 | — | — | 0.624 |
| | 96 | — | — | — | — | 0.071 | 0.115 | 0.036 |
| | 100 | 0.830 | — | — | — | — | — | 0.304 |
| | 102 | — | — | — | — | 0.929 | 0.885 | — |
| | 106 | 0.170 | — | — | — | — | — | — |
| N | | 56 | 28 | 37 | 20 | 14 | 26 | 28 |
| Ex | | | | | | | c | a |
| *f* | | | | | | | 0.635 | 0.664 |
| *Mdh*-1 | 70 | — | — | — | nd | — | — | 0.888 |
| | 95 | — | — | 0.039 | nd | — | — | — |
| | 97 | — | — | 0.039 | nd | — | — | 0.056 |
| | 100 | 0.964 | — | — | nd | — | 1.000 | — |
| | 102 | — | 1.000 | — | nd | 1.000 | — | 0.056 |
| | 104 | — | — | 0.870 | nd | — | — | — |
| | 107 | 0.036 | — | 0.026 | nd | — | — | — |
| | 110 | — | — | 0.013 | nd | — | — | — |
| | 117 | — | — | 0.013 | nd | — | — | — |
| N | | 82 | 70 | 38 | | 11 | 24 | 18 |
| Ex | | | | | | | | b |
| *f* | | | | | | | | 0.477 |
| **Mdh*-2 | 89 | — | — | — | 0.294 | — | 0.083 | — |
| | 92 | — | — | — | — | — | 0.042 | — |
| | 97 | — | — | — | — | — | 0.375 | — |
| | 100 | 0.987 | — | 1.000 | — | — | 0.083 | — |
| | 102 | — | 1.000 | — | — | — | 0.083 | — |
| | 105 | 0.013 | — | — | — | — | 0.313 | — |
| | 108 | — | — | — | — | 1.000 | — | — |
| | 111 | — | — | — | — | — | 0.021 | — |
| | 113 | — | — | — | 0.647 | — | — | — |
| | 118 | — | — | — | — | — | — | 0.280 |
| | 120 | — | — | — | — | — | — | 0.620 |
| | 122 | — | — | — | 0.059 | — | — | — |
| | 125 | — | — | — | — | — | — | 0.100 |
| N | | 83 | 70 | 59 | 17 | 9 | 24 | 25 |
| Ex | | | | | | | | b |
| *f* | | | | | | | | 0.410 |
| **Sdh* | 92 | — | — | 0.250 | — | — | — | — |
| | 95 | — | — | 0.406 | — | — | — | — |
| | 97 | 0.281 | — | 0.313 | — | — | — | 0.263 |
| | 100 | 0.719 | 1.000 | 0.031 | 1.000 | 0.100 | — | 0.737 |
| | 102 | — | — | — | — | 0.900 | 0.130 | — |
| | 107 | — | — | — | — | — | 0.566 | — |
| | 110 | — | — | — | — | — | 0.043 | — |
| | 113 | — | — | — | — | — | 0.152 | — |
| | 116 | — | — | — | — | — | 0.109 | — |
| N | | 16 | 16 | 16 | 16 | 10 | 23 | 19 |
| Ex | | | | | | | b | |
| *f* | | | | | | | 0.258 | |

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**Appendix 1.** *Continued.*

| Loci | Alleles | Species *Tph* | *Tde* | *Vau* | *Pun* | *Vve*# | *Cga*# | *Cch*# |
|---|---|---|---|---|---|---|---|---|
| **Pgm* | 87 | — | — | — | — | — | — | 0.038 |
| | 90 | — | — | 0.102 | — | — | — | 0.885 |
| | 93 | — | — | 0.078 | — | — | — | — |
| | 94 | — | — | 0.031 | 1.000 | — | 0.020 | 0.058 |
| | 95 | — | — | 0.031 | — | — | 0.020 | — |
| | 96 | — | — | 0.016 | — | — | — | 0.019 |
| | 97 | — | — | 0.101 | — | — | — | — |
| | 98 | 0.018 | 0.500 | 0.156 | — | — | 0.080 | — |
| | 100 | 0.389 | 0.392 | 0.228 | — | — | — | — |
| | 102 | 0.099 | 0.108 | 0.086 | — | — | 0.080 | — |
| | 104 | 0.414 | — | 0.101 | — | — | — | — |
| | 105 | — | — | 0.031 | — | — | 0.140 | — |
| | 108 | 0.080 | — | 0.039 | — | — | 0.220 | — |
| | 112 | — | — | — | — | 0.154 | 0.260 | — |
| | 114 | — | — | — | — | — | 0.040 | — |
| | 115 | — | — | — | — | 0.269 | — | — |
| | 116 | — | — | — | — | 0.077 | 0.080 | — |
| | 119 | — | — | — | — | 0.231 | — | — |
| | 120 | — | — | — | — | — | 0.060 | — |
| | 121 | — | — | — | — | 0.154 | — | — |
| | 125 | — | — | — | — | 0.077 | — | — |
| | 128 | — | — | — | — | 0.038 | — | — |
| N | | 81 | 60 | 64 | 18 | 13 | 25 | 26 |
| Ex | | | | | | d | | |
| *f* | | | | | | 0.281 | | |
| *6*Pgdh* | 82 | — | — | — | — | — | 0.020 | — |
| | 87 | — | — | — | — | — | — | — |
| | 89 | — | 0.082 | — | — | — | — | — |
| | 90 | — | — | 0.100 | — | 0.107 | 0.900 | — |
| | 93 | — | 0.031 | 0.127 | — | 0.393 | — | 0.058 |
| | 95 | 0.017 | — | — | — | — | 0.040 | — |
| | 96 | — | 0.122 | 0.292 | 0.289 | — | — | — |
| | 97 | 0.029 | — | — | — | 0.500 | — | 0.865 |
| | 98 | — | 0.286 | 0.118 | 0.527 | — | — | — |
| | 100 | 0.954 | 0.071 | 0.045 | 0.184 | — | 0.040 | — |
| | 102 | — | 0.204 | 0.146 | — | — | — | — |
| | 103 | — | — | — | — | — | — | 0.077 |
| | 104 | — | — | 0.109 | — | — | — | — |
| | 105 | — | 0.031 | — | — | — | — | — |
| | 107 | — | 0.173 | 0.027 | — | — | — | — |
| | 111 | — | — | 0.027 | — | — | — | — |
| N | | 86 | 49 | 55 | 19 | 14 | 25 | 26 |

*Tph*: *Tapes philippinarum*; *Tde*: *T. decussatus*; *Vau*: *Venerupis aurea*; *Pun*: *Paphia undulata*; *Vve*: *Venus verrucosa*; *Cga*: *Chamelea gallina*; *Cch*: *Callista chione*. #, new analysed samples. *, loci utilized for Nei distance calculation. N, number of analysed specimens. nd, no data. Ex, Exact test levels of significance: a, $P<0.001$; b, $P<0.01$; c, $P<0.02$; d, $P<0.05$. $f=F_{is}$ in Weir & Cockerham (1984) notation.



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