

Congruence and conflict in *Albinaria* (Gastropoda, clausiliidae). A review of morphological and molecular phylogenetic approaches

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ABSTRACT. *Albinaria* is a pulmonate genus distributed around the north-eastern coasts of the Mediterranean, exhibiting a high degree of morphological and molecular differentiation, especially in southern Greece and in the external insular Hellenic arc. As a consequence, traditional taxonomists have named more than 200 taxa (species and subspecies). Three major revisions indicate the complexity of this taxon and several authors have questioned the validity of its current taxonomy.

Recently, the use of both molecular and morphological phylogenetic approaches, on similar taxa sets, showed contradictory results. In cases where the palaeogeographic history of the area occupied by the studied taxa was unambiguous and the terminal taxa studied were relatively few, morphological and molecular results almost coincided. However, in cases where landscape modifications were drastic and unstable, and the number of terminal taxa was high, these approaches came to conflicting conclusions. The above situation indicates the methodological limitations of each approach and implies that evolutionary processes within *Albinaria* do not follow a stable and uniform (spatial and temporal) pattern.

In the present contribution the above mentioned contradictory results and methodological shortcomings are discussed, and a re-evaluation of characters and is proposed. I suggest that interpretation of results must be phylogenetically consistent and only monophyletic taxa should be accepted. Additionally, rate-dependent clustering or rooting methods which resulted in the more unresolved, unstable and unjustified inter- and intra-specific relations should be avoided. Unresolved, unstable and conflicting inter- and intra-specific topologies implied that certain characters must be re-evaluated or avoided in phylogenetic analyses for *Albinaria*. Such characters are: morphometrics, genitalia, certain highly homoplasious shell characters or allozyme loci, and non-conservative or ambiguously aligned genes

KEY WORDS: *Albinaria*, phylogeny, biogeography, morphology, molecular, phenetics, cladistics.

INTRODUCTION

Albinaria is a pulmonate genus distributed around the north-eastern coasts of the Mediterranean (Fig. 1), exhibiting a high degree of morphological and molecular differentiation, especially in southern Greece and in the external insular Hellenic arc. As a consequence, traditional taxonomists have named more than 200 taxa (species and subspecies). Several major revisions indicate the complexity of this taxon (BOETTGER, 1878; WAGNER,



Fig. 1. – The distribution of the genus *Albinaria* (shaded area).

1923, 1924; NORDSIECK, 1977, 1999) and several authors questioned the validity of its taxonomy (MYLONAS et al., 1987; MYLONAS, 1992; GIOKAS, 1996; DOURIS et al., 1998a). Beside mere taxonomic tasks, the study of *Albinaria* differentiation can be very informative about evolutionary processes because of the high species number that does not appear to be associated with a commensurate amount of ecological differentiation (GITTEBERGER, 1991), and of the complex palaeogeographic and climatic history of the Hellenic region (ANDEL & SHACKLETON, 1982; ANGELIER et al., 1982; BONNEAU, 1984; PAEPE, 1986; BERTOLDI et al., 1989; ANASTASAKIS & DERMITZAKIS, 1990; DERMITZAKIS, 1990).

Until 1980 the taxonomy of *Albinaria* was based on shell and lamellae morphology through the viewpoint of traditional taxonomy. However, more recently new approaches were followed using 1) new characters, such as: a) qualitative and quantitative morphological characters, b) allozymes, c) mtDNA, d) rRNA, and 2) modern analytical methods, such as: a) phenetics and b) cladistics (see Table 1). Now, there is fair account of information available, as several studied cases regarding phylogenetic relations in *Albinaria* have been published. However, almost no approach used all *Albinaria* species; usually they are restricted to the study of taxa within certain geographical regions of Greece.

The use of both molecular and morphological phylogenetic approaches on similar taxa sets led to contradictory results. Nevertheless, all of these approaches have certain merits and drawbacks. This situation is indicative of the methodological limitations of each approach. As HILLIS (1987) states "congruence between studies is strong evidence that the underlying historical pattern has been discovered, while conflict may indicate theoretical or procedural problems in one or both analyses, or it may indicate that additional data are needed to resolve the phylogenetic relationships in question".

The present study attempts to investigate a series of questions concerning the genus *Albinaria* that can be summarised as follows: a) which are the proposed phylogenetic relations between *Albinaria* species or monophyletic groups? b) are the results of phylogenetic analyses congruent with current taxonomy of the genus? c) which taxa appear to be paraphyletic or polyphyletic? d) how much effective is each approach in revealing robust phylogenetic relations? e) in what extent are the different approaches congruent?, and f) which are the evolutionary processes within *Albinaria*; is there a unique model of differentiation?

Therefore, in this study, these contradictory results and their shortcomings are presented, and the proposed evolutionary scenarios are discussed. An effective comparison of the various approaches concerning phylogenetic relations in *Albinaria* should inevitably take into consideration the fact that they dealt with sub-sets of *Albinaria* taxa, usually in the same biogeographical setting. Consequently, in order to compare the results of different studies these were divided into four categories corresponding to the following geographical regions of Greece: 1) Ionian islands, 2) Greek mainland, 3) Aegean region, 4) Crete. Finally, a last category, that is the whole of Greece, was added. For each approach the following data were recorded (Table 1): a) character type, b) analysis methods, c) number of species/populations studied.

The following criteria were applied for evaluating the effectiveness of each approach: a) representative sample of taxa and populations, b) statistically significant sample of specimens studied, c) stability of topologies, d) degree of homoplasy, e) concordance with geography and palaeogeography, and f) consistency of methodology. Finally, the results of these studies were collated and compared according to their corroboration in the corresponding phylogenetic hypotheses.

TABLE 1

Presentation of studies on *Albinaria* phylogeny with data on: a) character type, b) analysis methods, c) number species/populations studied, for each study.

| | Character type | Analysis methods | No of Species / populations studied |
|--------------------------------|---|----------------------|--|
| A. Ionian islands | | | |
| Kemperman, 1992 | • genitalia | • UPGMA & DAF | 4 / 12 |
| Kemperman et al., 1992 | • radula | • UPGMA & DAF | 4 / 13 |
| Kemperman & Degenars, 1992 | • allozymes | • UPGMA | 8 / 45 (3 <i>Albinaria</i> & 1 <i>Isabellaria</i> species from eloponnisos & Crete) |
| Kemperman & Gittenberger, 1992 | • shell qualitative-quantitative characters | • UPGMA & DAF | 4 / 12 |
| Giokas, 1996 | • shell qualitative characters | • parsimony analysis | 24 / 36 (5/8 Ionian taxa & the rest from mainland) |

| | Character type | Analysis methods | No of Species / populations studied |
|-----------------------------------|--|---|--|
| B. Greek mainland | | | |
| Schilthuizen et al., 1995 | • rRNA (ITS1) | • parsimony analysis | 5 / - (3 <i>Albinaria</i> & 1 <i>Isabellaria</i> species) |
| Giokas, 1996 | • shell qualitative characters | • parsimony analysis | 24 / 36 (1 <i>Sericata</i> & 3 <i>Isabellaria</i> species) |
| Douris et al., 1998b | • mtDNA (IrRNA) • shell qualitative characters | • neighbor-joining • parsimony analysis | 10 / - (1 <i>Clausilia</i> , 1 <i>Sericata</i> , 1 <i>Idyla</i> & 3 <i>Isabellaria</i> species) |
| C. Aegean region | | | |
| Douris et al. 1995 | • mtDNA (rest. site) • shell qualitative characters | • parsimony analysis | 3 / 10 (1 mainland species: <i>A. grisea</i>) |
| Giokas, 1996 | • shell qualitative characters | • parsimony analysis | 12 / 31 (outgroup: <i>Sericata sericata</i>) |
| Douris, 1997 | • mtDNA (COII) | • UPGMA • neighbor-joining • parsimony analysis | 7 / 22 (3 mainland populations of: <i>A. grisea</i> & <i>A. voithii</i>) |
| Douris, 1997 | • mtDNA (IrRNA) | • UPGMA • neighbor-joining • parsimony analysis | 10 / 54 (12 mainland populations of: <i>A. grisea</i> , <i>butoti</i> , <i>discolor</i> , <i>puella</i> , <i>voithii</i> & <i>Isabellaria</i>) |
| D. Crete | | | |
| Schilthuizen & Gittenberger, 1996 | • allozymes | • UPGMA • parsimony analysis | 13 / 38 (3 non-Cretan <i>Albinaria</i> & 1 <i>Isabellaria</i> species) |
| Giokas, 1996 | • shell qualitative characters | • parsimony analysis | 31 / 67 (outgroup: <i>Sericata sericata</i>) |
| Douris et al., 1998a | • mtDNA (IrRNA) | • neighbor-joining • parsimony analysis | 18 / 27 (1 non-Cretan <i>Albinaria</i> & 1 <i>Isabellaria</i> species) |
| E. Greece | | | |
| Ayoutanti et al., 1993 | • allozymes | • neighbor-joining | 15 / 31 |
| Ayoutanti, 1994 | • allozymes | • UPGMA | 15 / 31 |
| Giokas, 1996 | • shell qualitative characters | • parsimony analysis | 68 / - (outgroup: <i>Sericata sericata</i>) |
| Douris, 1997 | • mtDNA (IrRNA) | • UPGMA • neighbor-joining • parsimony analysis | 27 / 39 (outgroup: <i>Isabellaria saxicola</i>) |
| Douris et al., 1998a | • mtDNA (IrRNA) | • neighbor-joining • parsimony analysis | 23 / - (outgroup: <i>Isabellaria saxicola</i>) |

COMPARATIVE PRESENTATION OF RESULTS AND DISCUSSION

Ionian islands

The study of KEMPERMAN (1992) on two Ionian islands (Kephallinia and Ithaki) was the first attempt to investigate phylogenetic relations of *Albinaria* taxa (*A. senilis*, *A. adrianae*, *A. contaminata* and *A. jonica*) in this region. Kemperman studied several types of characters; genitalia,

radulae, allozymes, shell qualitative and quantitative characters (Table 1A). A series of important outcomes were revealed.

KEMPERMAN's (1992) results questioned the taxonomic importance of genitalia characters, as different groupings resulted according to the date of specimen collection (autumn, spring) and the method of analysis. Additionally, the distinction of nominal species was ambiguous (for example *A. senilis* and *A. adrianae* appeared to be polyphyletic).

KEMPERMAN et al. (1992) found strong indications for parallel evolution of radulae characters, and of correlation of teeth size with substrate. Therefore, radulae characters did not result to unambiguous nominal species distinction, as groupings of subspecies corresponded only partly with current taxonomy (only *A. contaminata* subspecies constituted a monophyletic group).

KEMPERMAN & DEGENAARS (1992) working with allozymes proposed the monophyly of the *Albinaria* species from Ionian islands, in respect to *Albinaria* species from Crete and Peloponnisos. Additionally, they found low genetic distances between *Albinaria* species from Ionian islands, as well as evidence for introgressive hybridisation. However, ambiguous species distinction resulted again, as only subspecies of *A. contaminata* constituted a monophyletic group.

KEMPERMAN & GITTENBERGER (1992) adopted the view that morphological characters represent two kind of components, viz., size and shape (HUMPREYS et al., 1981; POVEL, 1987). Size can be regarded as single scale factor and shape is composed of diverse factors. Their UPGMA analysis of dissimilarity matrices on size and shape components of morphometric characters resulted in different dendrograms according to "size" and "shape" differences.

Therefore, there were several drawbacks concerning the efficiency of these studies to reveal phylogenetic relations in *Albinaria*, as genitalia, radulae, allozymes and morphometry led to different groupings of the studied *Albinaria* taxa. However, it is surprising that when KEMPERMAN & GITTENBERGER (1992) combined (Dissimilarity matrix of All Factors-DAF) some sets of all the above characters types (genitalia, radulae, shell, and genetic distances), an unexpected distinction of nominal *Albinaria* taxa, concordant with current taxonomy, was obtained. Nevertheless, the use of rate-dependent clustering methods (UPGMA, DAF), puts into question the validity of the latter result.

GIOKAS (1996) studied all *Albinaria* nominal species from Ionian islands analysing shell qualitative characters via parsimony analysis. The results of this study confirmed the monophyly of each Ionian *Albinaria* species. Additionally, the revealed inter-specific relations did not support either NORDSIECK (1977, 1999) or KEMPERMAN & GITTENBERGER (1992), besides the fact that *A. contaminata* was found to be the more distant relative within Ionian *Albinaria* taxa. However, intra-specific topologies were rather unstable, as relatively high homoplasy was observed.

Greek mainland

There are few approaches concerning exclusively *Albinaria* taxa from the Greek mainland. Usually, with the exception of GIOKAS (1996), these studies used only a small portion of nominal *Albinaria* species. Additionally, they included certain *Isabellaria* taxa and populations, in

an effort to investigate the questionable monophyly of the genus *Isabellaria* (GITTENBERGER, 1994).

All studies resulted to the same major conclusion, that *Isabellaria* is a polyphyletic genus. Certain supposedly *Isabellaria* species from Peloponnisos are placed within *Albinaria*, e.g. *I. haessleini* (SCHILTHUIZEN et al., 1995; GIOKAS, 1996; DOURIS et al., 1998b), *I. butoti* and *I. campylauchen* (GIOKAS, 1996; DOURIS et al., 1998b). There is a partial concordance between morphological and molecular results. In Douris et al. (1998b), the exclusion of certain morphological characters associated with the GCA clausilium (an homoplastic complex of lamellae (SCHILTHUIZEN et al., 1995; DOURIS et al., 1998b) from the analysis led to almost identical morphological and molecular topologies.

In some cases, however, inter- and intra-specific relations were unstable (GIOKAS, 1996; DOURIS et al., 1998b) or unresolved (GIOKAS, 1996), probably due to the homoplasy of several studied characters. This is a problem resulting from all character sets, but in the case of mtDNA and rRNA, because of the small number of taxa studied and their distant relationships, did not result to considerable drawbacks, as for example in GIOKAS (1996), where a relatively large number of populations and species of *Albinaria* was studied. However, GIOKAS (1996) succeeded to identify certain monophyletic *Albinaria* groups in this region, usually congruent with nominal taxa. Additionally, *Albinaria* taxa from Kythira and Antikythira (*A. cytherae*, *A. grayana*, *A. compressa*) constituted a monophyletic group which is a sister group to certain south-east Peloponnisian *Albinaria* taxa (*A. discolor*, *A. voithii*, *A. haessleini*).

Finally, according to GIOKAS (1996) differentiation of the genus *Albinaria* in this region could be biogeographically explicable in terms of a vicarianistic model partly consistent with the palaeogeography of this region (ANDEL & SHACKLETON, 1982), as there is a clear separation of *Albinaria* taxa from west Greece, as well as those from KYTHIRA-ANTI KYHIRA island group, and certain taxa from the formerly temporally isolated south Argolis (*A. mixta*, *A. profuga*) constitute a monophyletic group. Nevertheless, this pattern is modified by probable secondary dispersal phenomena. Consequently, it is obvious that more research is needed in order to resolve phylogenetic relations, especially between *Albinaria* taxa from Peloponnisos.

Aegean region

Several approaches (see Table 1 and below) have been attempted to investigate phylogenetic relations between *Albinaria* taxa distributed in this region. However, they usually use a small number of the nominal taxa of this area, probably with the exception of GIOKAS (1996).

The monophyly of certain nominal taxa (*A. discolor*, *A. turrita*, *A. puella*) is confirmed. However, there are some

notable exceptions where current taxonomy is disputed. Namely, in all analyses (AYOUTANTI et al., 1993; GIOKAS, 1996; DOURIS, 1997) two nominal species, *A. coerulea* and *A. brevicollis*, appear to be polyphyletic. However, when their populations were considered together they constitute a definite monophyletic group and consequently these two species are lumped. Another case of lumping concerns *A. unicolor* and *A. olivieri* (GIOKAS, 1996) two taxa distributed on the Karpathos-Kasos island group.

DOURIS et al., (1995) estimated a nucleotide divergence rate of about 5%/myr. Additionally, DOURIS, (1997), according to mtDNA results, supports that intra-specific differentiation is lower within island than mainland *Albinaria* species and that there is a strong indication for star phylogeny.

However, certain inter-and intra- specific relations are often unstable. There is no general agreement concerning inter- and intra-group topologies. In mtDNA analyses different topologies arise depending on the gene studied (COII or IrRNA), or on the methods of analysis (UPGMA, neighbor-joining, parsimony). Especially, phenetic methods resulted to unexpected, or biogeographically unjustified groupings (e.g. topology of *A. puella*, *A. lerosiensis* and *A. voithii*). Topologies resulting from different data sets (shell characters and mtDNA) conform only if the studied taxa and populations are few, as in DOURIS et al., (1995). Probably, instabilities are due to the insufficient number of specimens studied resulting to unknown intra-population variation. Additionally, it seems that when more than few populations are studied, the stability of the topologies is affected by the conservative nature of the studied genes, e.g. the less conservative COII yields more unstable topologies than the IrRNA. In that way the limitations of each approach are indicated.

The more coherent topology, in terms of its agreement with the palaeogeography of this area, comes from the parsimony analysis of shell qualitative characters (GIOKAS, 1996), besides the relatively high homoplasy observed. This topology definitely supports a vicarianistic model of differentiation of *Albinaria* in this region consistent with the sequence of the palaeogeographic events of this region (ANGELIER et al, 1982; ANASTASAKIS & DERMITZAKIS, 1990; NOOMEN et al, 1996; REILINGER et al, 1997). There is a clear distinction of the monophyletic group of north-eastern Aegean taxa (*A. cristatella*, *A. puella*, *A. mitylena*), followed by the separation of one mainland species (*A. discolor*). Finally, there is a separation of southern and eastern Aegean *Albinaria* taxa (*A. munda*, *A. coerulea*, *A. turrita*, *A. olivieri*, *A. unicolor*). Within the latter there is a distinction between east and west distributed species.

Crete

Results from approaches concerning Cretan *Albinaria* taxa, have the most problematic interpretations, are the

more diverse, and generally reveal strong incongruence. The monophyly of Cretan taxa is either disputed (AYOUTANTI et al., 1987; DOURIS et al., 1998a) or favoured (SCHILTHUIZEN & GITTEBERGER, 1996). Paraphyletic or polyphyletic nominal species are common place, and all researchers agree that the current taxonomy does not reflect phylogeny accurately.

More specifically, SCHILTHUIZEN & GITTEBERGER (1996), working with allozymes, support the monophyly of Cretan taxa using parsimony analysis (using one *Isabellaria* species and 3 mainland *Albinaria* species as outgroup). Additionally, they confirm the monophyly of certain nominal species (*A. spratti*, *A. idaea*). However, in their UPGMA analysis, the outgroup mainland taxon *A. scopulosa* clusters within Cretan species. Moreover, the two kinds of analysis differ in the revealed inter- and intra-group topologies. Probably, this suggests that UPGMA suffers from its assumptions that evolution rates are homogenous. However, between islands and the mainland, there good reasons for rates being different. In spite of the fact that certain nominal species proved to be polyphyletic (*A. cretensis*, *A. hippolyti*), or paraphyletic (*A. corrugata*, which contains *A. moreletiana*), SCHILTHUIZEN & GITTEBERGER (1996) support the view that especially the latter does not consist a major taxonomic problem (as hard cladistic theory supports), and they claim that paraphyletic species can be considered as natural phenomena. These authors justify their opinion by proposing that isolated populations, resulting from the accidental colonization of single patches of suitable habitat, or being relicts of former, larger areas of distribution, may be regarded as genetically cut off from others. Such isolates, due to sufficient natural selection, genetic drift and/or founder effect, may move away from their original, balanced phenotype, and evolve into new species. Nevertheless, this argument, as the above authors admit, is weakened by the fact that the sample of *A. corrugata* to which *A. moreletiana* appears most closely related, originates from a distant part of *A. corrugata* range. However, these authors dispute the current taxonomy in the case of the polyphyletic *A. cretensis*.

DOURIS et al. (1998a), studying about half of the Cretan nominal species, support the non-monophyly of Cretan *Albinaria* species, as certain non-Cretan species from the Aegean region (*A. lerosiensis*, *A. coerulea*, *A. turrita*) or the Greek mainland (*A. discolor*, *A. grisea*, *A. butoti*) are placed within species from Crete. However, the revealed inter-group topologies have a low bootstrap support, and intra-group topologies arising from parsimony and neighbor-joining analysis differ in certain cases. DOURIS et al. (1998a) provide a biogeographic explanation for phylogenetic relations within Cretan taxa, supporting that there is a macro-geographical pattern of distribution of clusters along an east-west axis of the island. However, they either avoid to explain in biogeographic and palaeogeographic terms certain problematic topologies e.g. the position of mainland (*A. discolor*, *A. grisea* and *A. butoti*) and

Aegean taxa (*A. coerulea*, *A. turrita*) within Cretan taxa, or when they do, as their estimation of 1-1.2% differentiation rate per myr, they base their arguments (in the case of *A. lerosiensis*) on dubious palaeogeographic data (see DERMITZAKIS, 1987; ANASTASAKIS & DERMITZAKIS, 1990; WESTEWAY, 1994), such as former connections of Dodekanissa with Crete. Finally, these authors identify certain monophyletic (*A. hippolyti*, *A. spratti*, *A. corrugata*, *A. torticollis*) and paraphyletic (*A. praeclara*, *A. eburnea*) nominal species.

AYOUTANTI et al. (1993), working on allozymes, provide intermediate results concerning the monophyly of Cretan *Albinaria* taxa. In their mid-point rooted neighbor-joining tree, *A. lerosiensis* is also placed within Cretan taxa, while *A. grisea*, *A. discolor*, *A. coerulea* and *A. turrita* are not.

GIOKAS (1996), determined certain monophyletic (*A. hippolyti*, *A. corrugata*, *A. eburnea*, *A. sublamellosa*, *A. violacea*, *A. manselli*, *A. torticollis*), paraphyletic (*A. teres*) and polyphyletic (*A. cretensis*, *A. idaea*) nominal *Albinaria* species, questioning, in several cases, the current taxonomy and the monophyly of Cretan taxa. However, the highest levels of homoplasy and many unresolved (hard or soft polytomies) and unstable topologies were observed in Cretan taxa. Probably, this was due to the large number of populations studied, or it may reflect more complex evolutionary phenomena. GIOKAS (1996) provided a partial geographic (East-West axis distinction of *Albinaria* groups in Crete), and palaeogeographic interpretation (proposal for relict species). However, in central Crete, where accurate palaeogeographic data are poor, and ecological and landscape alterations are frequent and drastic, it seems that secondary dispersal phenomena can modify unpredictably this evolutionary scenario, resulting to "noisy" information.

Greece

Analogous extreme discordance is apparent among analyses containing taxa from all over Greece. The neighbor-joining results of AYOUTANTI et al. (1993) suggest the distinction of mainland, island and Cretan species, with the exception of the insular species *A. lerosiensis* (Kos island) and *A. cytherae* (Antikythira island) that are clustered together with Cretan species. UPGMA results of AYOUTNANTI (1994) showed more complex groupings. Results of DOURIS (1997) support the non-monophyly of taxa distributed to adjacent regions and even though groupings from prior analyses are retained, high instability arises (low bootstrap values), and different analysis methods (UPGMA, neighbor-joining, parsimony) produce different inter- and intra-group topologies. DOURIS (1997) explains the low bootstrap values near the roots of the dendrograms by suggesting that at the first stages of the *Albinaria* evolution, differentiation was rapid (star phylogeny), and he does not discuss the option of methodological shortcomings. On the contrary, GIOKAS (1996)

disputes the validity even of his own results, and suggests that in front of extreme unstable topologies phylogenetic interpretations are doubtful.

CONCLUSIONS

Both morphological and molecular systematic approaches have certain advantages for phylogenetic reconstruction. Thereby, studies that combine them can increase information content and utility. However, it is important to choose analysis methods that are as possible assumption-free and responsive to combination of data sets. This requires rate-independent methods of tree construction and rooting, as well as use of character-state data rather than distance summaries whenever possible (HILLIS, 1987). Such combinations of molecular and morphological studies could provide a comprehensive view of biotic evolution.

Nevertheless, all of these approaches on *Albinaria* phylogeny appear to have certain merits and drawbacks. The use of both molecular and morphological approaches on similar taxa sets showed contradictory results. The above situation is indicative of the methodological limitations of each approach and implies that evolutionary processes within *Albinaria* do not follow a stable and uniform (spatial and temporal) pattern. In cases where the palaeogeographic history of the area occupied by the studied taxa was unambiguous and the terminal taxa studied were relatively few, morphological and molecular results coincided. However, in cases where landscape modifications were drastic and unstable, and the number of terminal taxa was high, these approaches came to conflicting conclusions.

More specifically I suggest that: (1) Phylogenetic relations within mainland and Ionian *Albinaria* taxa are partly congruent with the palaeogeography of this area. Dispersal, or secondary contact phenomena have a useful supplemental explanatory role. Phylogenetic analyses result in conflicting results. However the validity of UPGMA results should be treated with caution. (2) The monophyly of most nominal species in the Aegean region, and the partial concordance (in most analyses) of their phylogenetic relations with area's geography and palaeogeography, favours the hypothesis of a vicarianistic differentiation as the most probable evolutionary scenario for Aegean *Albinaria* taxa. (3) Different analyses resulted in significantly different topologies concerning Cretan *Albinaria* taxa. Questionable and unstable inter- and intra-group polyphyletic or paraphyletic topologies among Cretan *Albinaria* taxa imply complex evolutionary processes, resulting partly from vicarianistic phenomena, followed by secondary contact and introgression, as well as by species or populations range expansion and contraction. (4) *Isabellaria* is a polyphyletic genus, as in all analyses several former *Isabellaria* taxa are grouped within certain *Albinaria* species.

However, an overall resolution of the phylogenetic relations within *Albinaria* has not been achieved yet due to: 1) high homoplasy in certain cases, 2) certain morphological character incompatibility, 3) uncertainty in homologizing allozyme variants, 4) insufficient calibration and drawbacks of molecular techniques, 5) limitations of computing algorithms, 6) fragmented palaeogeographic and palaeoecological information.

Moreover, I support that interpretation of the results must be phylogenetically consistent. Consequently, lumping of *Albinaria* species forming robust monophyletic groups, and splitting polyphyletic nominal species should be favoured. As far as it concerns paraphyletic "species" several authors suggest that paraphyletic "species" may be a general phenomenon in nature (HAFNER et al, 1987; DE QUEIROZ & DONOGHUE, 1988; SCHILTHUIZEN & GITTEBERGER, 1996). However, I do not agree with the viewpoint of SCHILTHUIZEN & GITTEBERGER, (1996) that paraphyletic species are not evidence for incorrect taxonomy. Nominal species must not be accepted a priori. Robust parsimony analyses can identify paraphyletic "species". That means that formerly named species found to be placed within other species should not retain their former species status because in cladistic terms a species must be monophyletic.

Finally, rate-dependent clustering or rooting methods result in the more unresolved, unstable and unjustified inter- and intra-specific relations and should be avoided in phylogenetic analyses. Unresolved, unstable and conflicting inter- and intra-specific topologies imply that certain characters must be re-evaluated or avoided in phylogenetic analyses for *Albinaria*. Such characters are: morphometrics, genitalia, certain highly homoplasious shell characters or allozyme loci, and non-conservative or ambiguously aligned genes.

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REFERENCES

- ANASTASAKIS, G.C. & M. DERMITZAKIS (1990). Post-Middle-Miocene paleogeographic evolution of the Central Aegean Sea and detailed Quaternary reconstruction of the region. Its possible influence on the distribution of the Quaternary mammals of the Cyclades Islands. *Neues Jahrbuch, Geologie und Paleontologie Monatsheft*, 1: 1-16.
- ANDEL, Van T.H. & J.C. SHACKLETON (1982). Late Paleolithic and Mesolithic coastlines of Greece and the Aegean. *Journal of Field Archaeology*, 9: 445-454.
- ANGELIER, J., N. LYMBERIS, X. LE PICHON, E. BARRIER & P. HUCHON (1982). The tectonic development of the Hellenic arc and sea of Crete: A synthesis. *Tectonophysics*, 86: 159-196.
- AYOUTANTI, A. (1994). *Geographic structure of genetic variation of populations of genus Albinaria in the region of the Aegean archipelago*. Ph.D. thesis. Agricultural Univ. of Athens, Greece.
- AYOUTANTI, A., C. KRIMBAS, S. TSAKAS & M. MYLONAS (1987). Genetic differentiation and speciation in the Greek archipelago: The genus *Albinaria*. *Biologia Gallo-hellenica*, 13: 155-160.
- AYOUTANTI A., C. KRIMBAS, J. SOURDIS & M. MYLONAS (1993). Geographic structure of the genus *Albinaria* as revealed by electrophoretic genetic data. *Biologia Gallo-Hellenica*, 20: 19-23.
- BONNEAU, M. (1984). Correlation of the Hellenides nappes in the south-east Aegean and their tectonic reconstruction. *Geol. Soc. London, sp. publ.*, 17: 517-527.
- BERTOLDI, R., D. RIO & R. THUNELL (1989). Pliocene-Pleistocene vegetational and climatic evolution of the south-central Mediterranean. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 72: 263-275.
- BOETTGER, O. (1878). Monografie der Clausiliensektion *Albinaria* v. Vest. *Novitates Conchologicae* 1 Abteilung, 5: 39-173 Cassel.
- DE QUEIROZ, K. & M.J. DONOGHUE (1988). Phylogenetic systematics and the species problem. *Cladistics*, 4: 317-338.
- DERMITZAKIS, M.D. (1987). *General introduction to the geology of Crete (field guide for the excursion)*. University of Vienna.
- DERMITZAKIS, M.D. (1990). Biogeographical aspects of insularity. *Atti Convegno Lincei*, 85: 263-288.
- DOURIS, V. (1997). *Molecular analysis of mtDNA of the genus Albinaria (Mollusca). Contribution to the study of molecular evolution and phylogeography of the genus in the Hellenic region*. Ph.D. thesis, University of Athens, Athens.
- DOURIS, V., G.C. RODAKIS, S. GIOKAS, M. MYLONAS & R. LECANIDOU (1995). Mitochondrial DNA and morphological differentiation of *Albinaria* populations (Gastropoda: Clausiliidae). *Journal of Molluscan Studies*, 61: 65-78.
- DOURIS, V., R.A.D. CAMERON, G.C. RODAKIS & R. LECANIDOU (1998a). Mitochondrial phylogeography of the land snail *Albinaria* in Crete: Long-term geological and short-term vicariance effects. *Evolution*, 52(1): 116-125.
- DOURIS, V., S. GIOKAS, R. LECANIDOU, M. MYLONAS & G.C. RODAKIS (1998b). Phylogenetic analysis of mitochondrial DNA and of morphological characters suggest a need for taxonomic re-evaluation within the Alopinae (Gastropoda: Clausiliidae). *Journal of Molluscan Studies* 64: 81-92.
- GIOKAS, S. (1996). *The differentiation of the genus Albinaria in the Hellenic Region*. Ph.D. thesis, University of Athens, Athens.
- GITTEBERGER, E. (1991). What about non-adaptive radiation? *Biological Journal of the Linnean Society*, 43: 263-272.
- GITTEBERGER, E. (1994). Five new *Albinaria* subspecies from the eastern Peloponnese, Greece; with notes on *Isabellaria* s.l. (Gastropoda: Pulmonata: Clausiliidae). *Basteria*, 58: 55-62.
- GITTEBERGER, E. & M. SCHILTHUIZEN (1996). *Parallelism in the origin of the G-type clausilian apparatus (Gastropoda, Pulmonata, Clausiliidae)*. In: Taylor, J. D. (ed). *Origin and Evolutionary Radiation of the Mollusca*, Oxford University Press, London: 295-300.

- HAFNER, M.S., J.C. HAFNER, J.L. PATTON & M.F. SMITH (1987). Macrogeographic patterns of genetic differentiation in the pocket gopher *Thomomys umbrinus*. *Syst. Zool.*, 36: 18-34.
- HILLIS, D.M. (1987). Molecular versus morphological approaches to systematics. *Annu. Rev. Ecol. Syst.*, 18: 23-42.
- HUMPHREYS, J.M., F.L. BOOKSTEIN, B. CHERNOFF, G.R. SMITH, R.L. ELDERAND & S.G. POSS (1981). Multivariate discrimination by shape in relation to size. *Syst. Zool.*, 30: 291-308.
- KEMPERMAN, T.C.M. (1992). *Systematics and evolutionary history of the Albinaria species from the Ionian islands of Kephallinia and Ithaca (Gastropoda, Pulmonata: Clausiliidae)*. Ph.D. thesis, Barkhuys, Leiden.
- KEMPERMAN, T.C.M. & G.H. DEGENAARS (1992). Allozyme frequencies in *Albinaria* (Gastropoda Pulmonata: Clausiliidae) from the Ionian islands of Kephallinia and Ithaca. *Malacologia*, 34: 33-61.
- KEMPERMAN, T.C.M. & E. GITTENBERGER (1988). On morphology and taxonomic importance of the shell ribs in Clausiliidae (Mollusca: Gastropoda Pulmonata), with special reference to those of *Albinaria*. *Basteria*, 52: 77-100.
- KEMPERMAN, T.C.M. & E. GITTENBERGER (1992). *The genus Albinaria (Gastropoda Pulmonata: Clausiliidae) in Kephallinia and Ithaca (Ionian islands, Greece): an integrated approach*. In: T.C.M. Kemperman (ed.) Systematics and evolutionary history of the *Albinaria* species from the Ionian islands of Kephallinia and Ithaca (Gastropoda Pulmonata: Clausiliidae). Universal Book Services, Leiden: 159-233.
- KEMPERMAN, T.C.M., G.D.E. POVEL, & P.H.F. BOR (1992). *Differentiation in radulae of closely related pulmonates: a pilot study in Albinaria (Gastropoda: Clausiliidae)*. In: T.C.M. Kemperman (ed.) Systematics and evolutionary history of the *Albinaria* species from the Ionian islands of Kephallinia and Ithaca (Gastropoda Pulmonata: Clausiliidae) Universal Book Services, Leiden: 81-120.
- MYLONAS, M., C. KRIMBAS, S. TSAKAS, & A. AYOUTANTI (1987). The genus *Albinaria* Vest. (Clausiliidae, Gastropoda). Is there any true species? *Biologia Gallo-hellenica*, 13: 161-164.
- NOOMEN, R., T.A. SPRINGER, B.A.C. AMBROSIOS, K. HETZBERGER, D.C. KUIJPER, G.J. METS, B. OVERGAAUW & K.F. WALKER (1996). Crustal deformations in the Mediterranean area computed from SLR and GPS observations. *J. Geodyn.*, 21: 73-96.
- NORDSIECK, H. (1977). Zur Anatomie und Systematik der Clausilien, XVII. Taxonomische Revision des Genus *Albinaria* Vest. *Archives fur Molluskenkunde*, 107: 285-307.
- NORDSIECK, H. (1999). Annotated check-list of the species of the *Albinaria-Isabellaria* group (Gastropoda: Stylomatophora: Clausiliidae). *Mitt. Dtsch. Malakozool. Ges.*, 62/63: 1-21.
- PAEPE, R. (1986). Landscape changes in Greece as a result of changing climate during the Quaternary. In: FANTECHI, R. & N.S. MARGARITIS (eds). *Desertification in Europe*: 49-58.
- POVEL, G.D.E. (1987). Pattern detection within *Ypononeuta padellus*-complex of the European small ermine moths (Lepidoptera, Yponomeutidae). II. Procedural conditions for the estimation of shape-differences. *Proc. Kon. Akad. Wetensch.*, C, 90: 367-386.
- REILINGER R., S. MCCLUSKY, B. ORAL, R. KING, M.N. TOKSOZ, A. BARKA, I. KINIK, O. LENK & I. SANLI (1997). Global positioning system measurements of present-day crustal movements in the Arabia-Africa-Eurasia plate collision zone. *J. Geophys. Res.*, 102: 9983-9999.
- SCHILTHUIZEN, M. & E. GITTENBERGER (1996). Allozyme variation in some Cretan *Albinaria* (Gastropoda): Paraphyletic species as natural phenomena. In: TAYLOR, J. (ed.), *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, Oxford: 301-311.
- SCHILTHUIZEN, M., E. GITTENBERGER & A.P. GULTYAEV (1995). Phylogenetic relationships inferred from the sequence and secondary structure of ITS1 rRNA in *Albinaria* and putative *Isabellaria* species (Gastropoda, Pulmonata, Clausiliidae). *Molecular Phylogenetics and Evolution*, 4: 457-462.
- WAGNER, A.J. (1923). Ergänzungen und Erläuterungen zur Systematik der Clausiliiden. II. Neue Formen und Arten des Genus *Albinaria* ex. rect. mea. *Annales Zoologici Musei Polonici Historiae Naturalisi*, 2(1): 1-8.
- WAGNER, A.J. (1924). Ergänzungen und Erläuterungen zur Systematik der Clausiliiden. II. Neue Formen und Arten des Genus *Albinaria* ex. rect. mea. *Annales Zoologici Musei Polonici Historiae Naturalisi*, 2(2): 9-23.
- WESTEWAY, R. (1994). Present-day kinematics of the Middle East and eastern Mediterranean. *J. Geophys. Res.*, 99: 12071-12090.