Biodiversity Journal, 2012, 3 (3): 251-258

Additional data on the genus *Muticaria* Lindholm, 1925 with description of a new species (Gastropoda Pulmonata Clausiliidae)

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ABSTRACT Morphological analysis and molecular genetic studies conducted on the genus *Muticaria* Lindholm, 1925 (Pulmonata Clausiliidae) in Sicily allowed to identify a new species which is described in the present paper.

KEY WORDS Clausiliidae; *Muticaria*; Sicily, new species.

Received 01.09.2012; accepted 18.09.2012; printed 30.09.2012

INTRODUCTION

The genus *Muticaria* Lindholm, 1925 has a distribution limited to South-East Sicily and Maltese Islands. Currently it includes three species: *Muticaria syracusana* (Philippi, 1836) and *M. neuteboomi* Beckmann, 1990 spread in southeastern Sicily and *M. macrostoma* endemic to the Maltese Islands, where it occurs with four subspecies: *M. macrostoma macrostoma* (Cantraine, 1835), *M. macrostoma scalaris* (L. Pfeiffer, 1850), *M. macrostoma oscitans* (Charpentier, 1852) and *M. macrostoma mamotica* (Gulia, 1861) (Beckmann, 1992; Giusti et al.,1995; Bank, 2012).

A preliminary molecular study on 16S rDNA partial sequences (Gregorini et al., 2008) carried out on Sicilian *Muticaria* revealed the existence of significant genetic differences between populations attributed either to *M. syracusana* or *M. neuteboomi*, including the topotypic ones.

Particularly, *M. neuteboomi* resulted the most widespread species with populations inhabiting inner areas of Iblean plateau (South Eastern Sicily), while *M. syracusana* resulted confined to a few coastal locality of Syracuse province.

A second and more detailed molecular study (Colomba et al., 2010) was conducted on topotypic specimens of *M. syracusana* and *M. neuteboomi* with a comparative analysis of mitochondrial 16S rDNA and cytochrome oxidase I (COI) gene partial sequences. This study, besides confirming preliminary data (Gregorini et al., 2008), strongly corroborated the validity of the two species.

As additional contribute to the research on the genus *Muticaria* in South Eastern Sicily and within the context of a wider and more detailed work, in the present paper the population of *Muticaria* from Spinagallo (Syracuse) is described as new species on the grounds of morphological and molecular data.

ACRONYMS. BC = bursa copulatrix; BCD = diverticulum of bursa copulatrix; CL = columellar lamella; DBC = duct of the bursa copulatrix; DE= distal epiphallus; FO = free oviduct; GA = genital atrium; L = lunella; P = penis; PD = diverticulum of penis; PE= proximal epiphallus; PL = parietal lamella; PLL = parallel lamella;

PP = principal plica; PR = penial retractor muscle; SL = spiral lamella; SP = sutural plica/plicae; V= vagina; VD = vas deferens.

The materials used for this study are deposited in the following Museums and private collections: A. Brancato collection, Syracuse, Italy (CB); S. Giglio collection, Cefalù, Italy (CG); Laboratory of Cytogenetics and Molecular Biology, University of Urbino, Italy (LCMBU); F. Liberto collection, Cefalù, Italy (CL); Museo Civico di Storia naturale di Comiso, Italy (MCSNC); Museo Civico di Storia Naturale di Genova "G. Doria", Italy (MSNG); Museo Naturalistico F. Minà Palumbo, Castelbuono, Italy (MNMP); A. Reitano collection, Tremestieri Etneo, Italy (CR); I. Sparacio collection, Palermo, Italy (CS).

Muticaria brancatoi n. sp.

Examined material. Holotypus: Italy, Sicily, Siracusa, Cugno Lungo, 37°00'25"N 15°10'47"E, 110 m, 02.IX.2012, legit A. Brancato (MSNG 57016). Paratypi: Italy, Sicily, Siracusa, Contrada Spinagallo, 37°00'12"N 15°10'50"E, 120 m, 12.III.2008, 5 specimens, 3 shells (CR); idem, 14 specimens, 30 shells (CR); Siracusa, V.ne Moscasanti, 37°00'58"N 15°09'53"E, 130 m, 28.XII.2010, 2 shells (CR); Siracusa, Cugno Lungo, 37°00'53"N 15°10'11"E, 135 m, 28.XII.2010, 2 specimens, 3 shells (CR) Siracusa, Cugno Lungo, 37°00'25"N 15°10'41"E, 110 m, 01.IV.2012, 16 shells (CL); Siracusa, Cugno Lungo, 37°00'27"N 15°10'48"E, 80 m, 01.IV.2012, 8 specimens, 86 shells (CL); idem, 2 specimens, 2 shells, legit F. Liberto (MCSNC 4412); idem, 6 shells (CG); Siracusa, Cugno Lungo, 37°00'25"N 15°10'47"E, 110 m, 02.IX.2012, 8 shells (CB); idem, 20 specimens, 32 shells (CS); idem, 2 specimens, legit I. Sparacio (MNMP).

Description of holotypus. Shell sinistral (Figs. 1, 2, 9), dimensions: height: 12.30 mm; maximum diameter: 4.20 mm, medium, cylindrical-fusiform, decollate, rather robust, light yellowish-grey in colour; external surface with minute, raised, close ribs, 69 ribs on penultimate whorl; last whorl with robust, evident and very spaced ribs; spire slowly and regularly growing, with 4 whorls; last whorl tapering downwards, with a very elevated and curved cervical keel; suture moderately deep; umbilicus slit-like, aperture about 1/3 of shell height, square, with 5 lamellae (on parietum and columellar side) and lunella and 4-5 plicae (on palatum); on parietum (Figs. 7, 8), starting from suture, there are: long, well developed, non-emerging parallel lamella; short spiral lamella, deviating from centre of parietum to adhere to parallel lamella, (upper) parietal lamella tooth-like; non-emerging columellar lamella; subcolumellar lamella internal; on palatum (Figs. 5, 6) there is an evident, lateral lunella and, starting from suture: two sutural plicae, the principal plica with a robust posterior portion, not fused to lunella apex, and a thin anterior portion, basal plica small, internally fused to base of lunella, very small sulcal lamella; clausilium triangular and slender (Figs. 3, 4), plough-like basal plate, apically pointed; peristome continuous, reflected, distinct from the wall of the last whorl.

Genitalia (Figs. 12-14). Genitalia are characterized by: short vagina, very short free oviduct, well developed ovispermiduct and a short copulatory duct ending in branched bursa copulatrix complex; one branch consisting of a short and wide diverticulum of the bursa copulatrix; other branch consisting of very short bursa copulatrix duct and oval and elongated bursa copulatrix. Penial complex consisting of flagellum, epiphallus, penial diverticulum and penis; epiphallus divided by point insertion of robust penial retractor muscle into proximal and distal portions, the latter very short; wide and pointed penial diverticulum arising on border between distal epiphallus and penis; penis short (2.5 mm). Internal walls of penis show a long, wide and elevated pleat and two thin and less evident pleats; left ommatophore long and well developed.

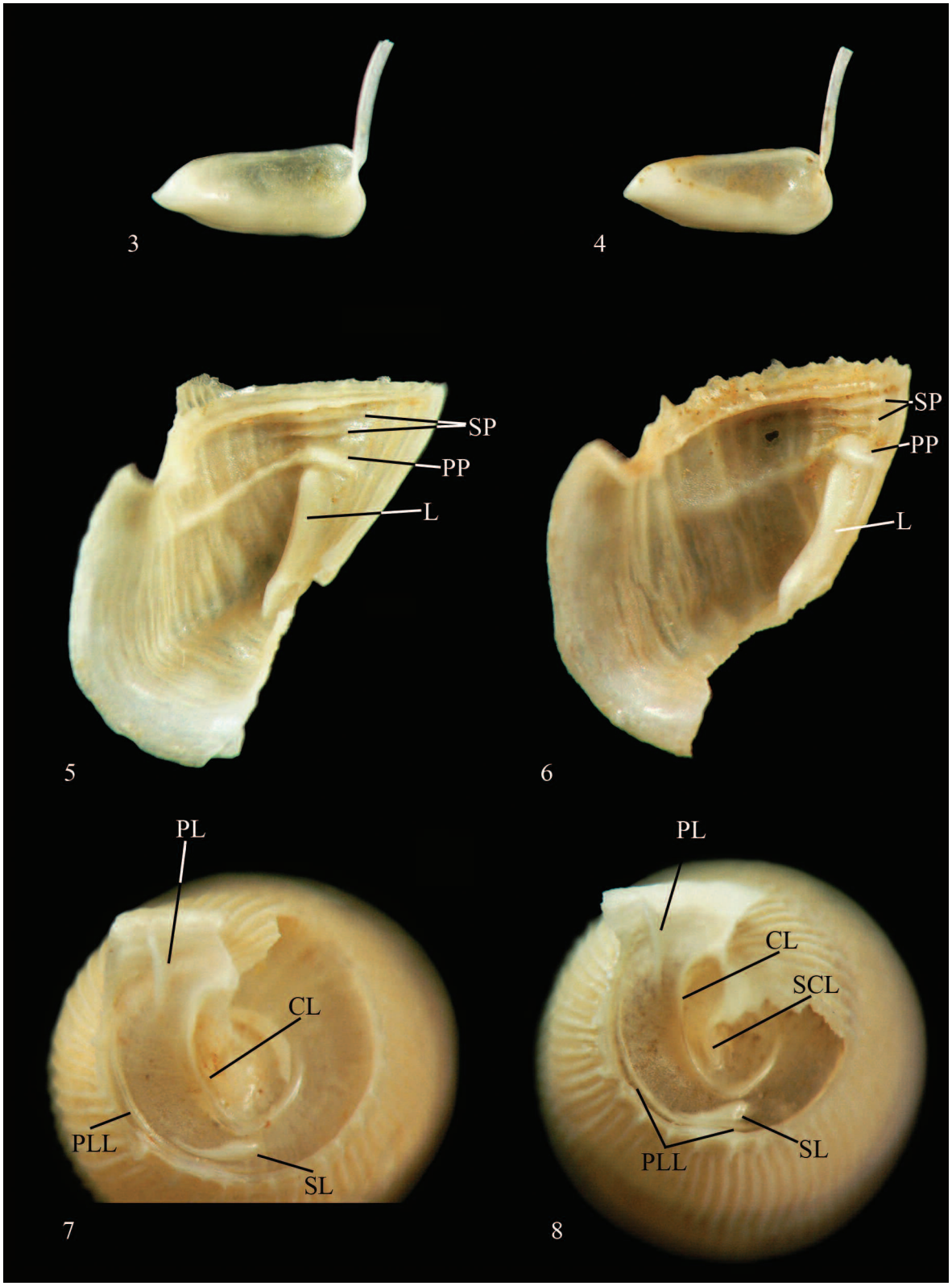
Variability. Dimensions in decollate specimens (4-5 whorls): height: 11.02-12.30 mm; maximum diameter: 4.16-4.55 mm. The number of ribs on the penultimate whorl of the shell ranges from 57 to 70 (on average, 67); in some specimens the principal plica is absent in its central portion.

Etimology. The new species is dedicated to Aldo Brancato (Syracuse, Sicily), dear friend and esteemed naturalist.

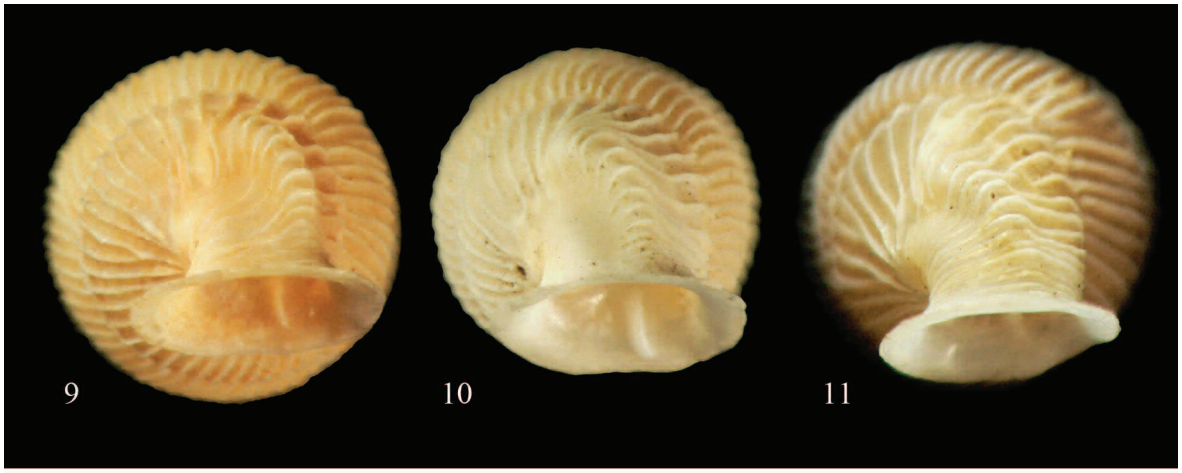
Biology and Distribution. This species lives on calcareous rock. It is found in cavities and under stone on stony soil. Endemic species to the South-Eastern Sicily, at the time known only for the locality of description.

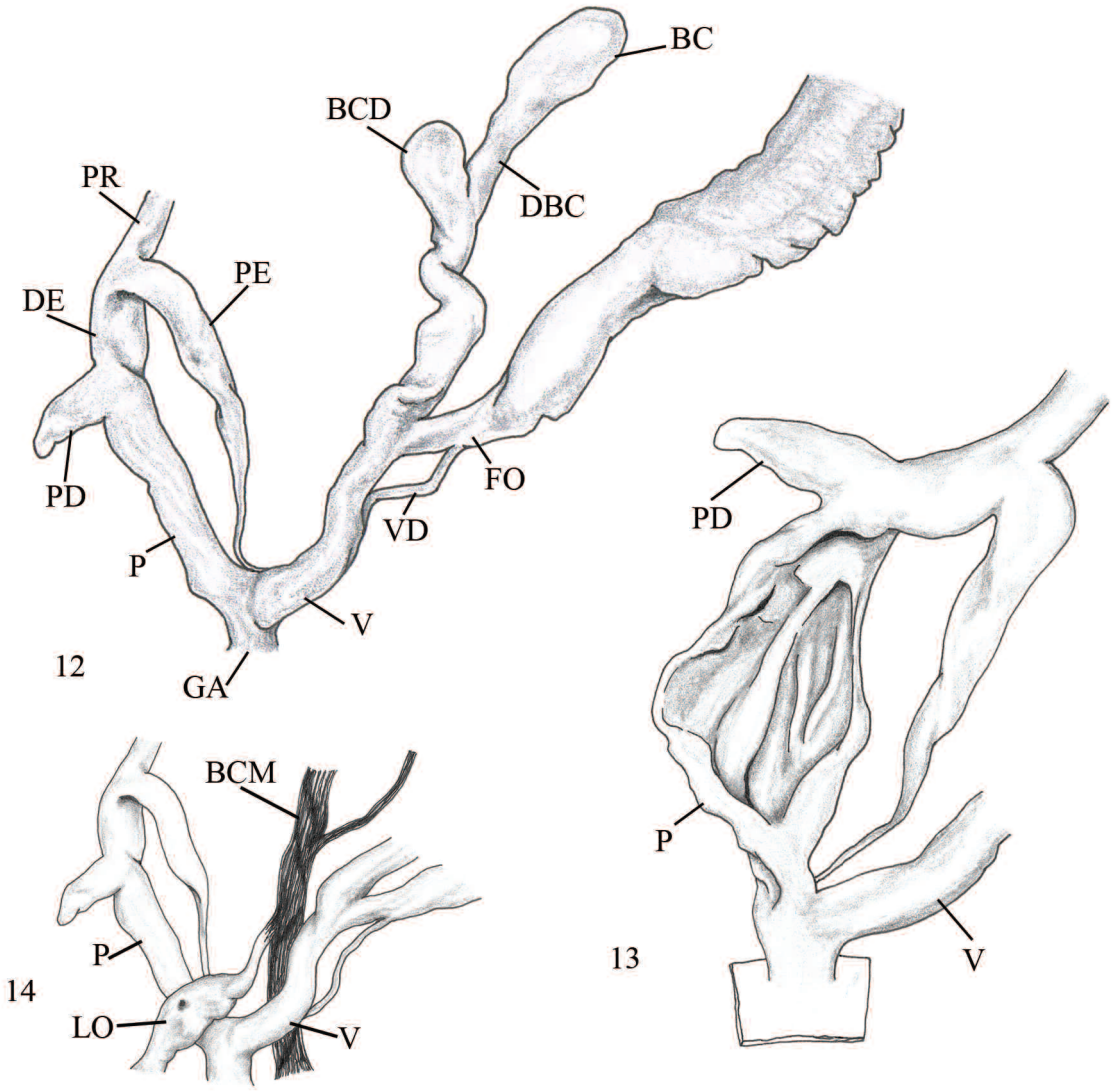
Comparative notes. *M. syracusana* shows slender and conical-fusiform shell with ribs on penulti-

Figure 1. Shell of *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo, h: 11.57 mm - D: 4.33 mm. Figure 2. idem, h: 12.27 mm - D: 4.29 mm.



Figures 3-8. *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo, clausilium of two specimens (Figs. 3, 4), palatum (Figs. 5, 6) and parietum (Figs. 7, 8).





Figures 9-11. Cervical keel in *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo (Fig. 9), *M. syracusana,* Siracusa, Teatro Romano (Fig. 10) and *M. neuteboomi,* Ragusa, Cava d'Ispica (Fig. 11). Figures 12-14. Genitalia of *M. brancatoi* n. sp., Siracusa, Cugno Lungo (Fig. 12) internal structure of penis (Fig. 13) and ommatophore (Fig. 14).

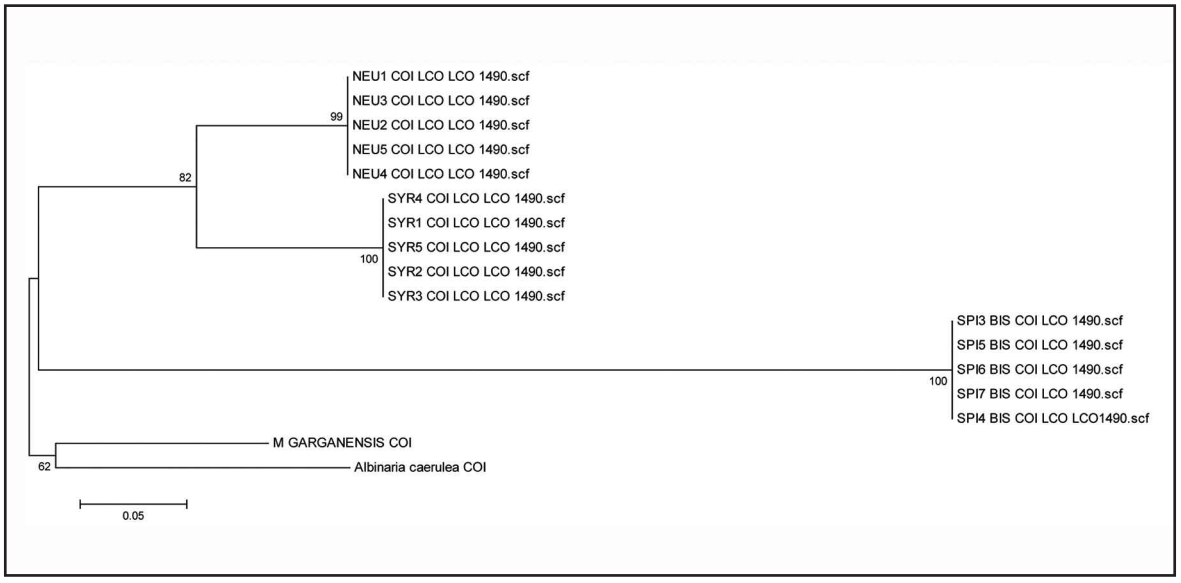


Figure 15. Maximum Likelihood consensus tree inferred from 500 replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values, i.e.the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches.

mate whorl more spaced and less numerous (27-54); on palatum, the principal plica is very short and fused to upper palatal plica. *M. neuteboomi* is characterized by fusiform shell, from slender to moderately ventricose, with more numerous ribs on penultimate whorl (56-97); on palatum, the principal plica is independent of upper palatal plica. All *Muticaria* from Maltese islands are characterized for a principal plica independent of the upper palatal plica.

Molecular Analysis. Five *Muticaria* specimens from C.da Spinagallo (Syracuse, SE Sicily), labelled as SPI, were analyzed. Samples were stored in 75% Ethanol at -20 °C in test tubes. For each individual, the entire animal was used for total DNA extraction (by Wizard Genomic DNA Purification Kit, Promega).

Para-voucher specimens, sensu Groenenberg et al. (2011) i.e. different specimens than the ones used for DNA analysis, but from the same sample or population, were stored by MSC (University of Urbino). COI amplicons (644 bp) were obtained by LCO1490/HCO2198 universal primers (5'-GGTCAACAAATCATAAAGATATTGG-3'/5'-TAAACTTCAGGGTGACCAAAAAATCA-3') as in Folmer et al. (1994) with a PCR cycle of 95 °C for 5 min; 95 °C for 1 min, 42 °C for 1 min, 72 °C for 1 min (37 cycles); 72 °C for 10 min. Sequencing of the purified PCR products was carried out using automated DNA sequencers at Eurofins MWG Operon (Germany). Finally, sequence chromatograms of each amplified fragment were browsed visually. Sequences generated in this study were analysed with additional *Muticaria syracusana* (labelled as SYR) and *M. neuteboomi* (labelled as NEU) COI sequences, previously deposited by us in GenBank (IDs: HQ696869 and HQ696867, see also Colomba et al., 2010) *Medora garganensis* (ID: AY425595) and *Albinaria caerulea* (ID: NC_001761) COI sequences were employed as outgropus.

Sequences were visualized with BioEdit Sequence Alignment Editor 7 (Hall, 1999), aligned with the ClustalW option included in this software and double checked by eye. Standard measures of nucleotide polymorphism and phylogenetic analyses were conducted in MEGA 5.0.3 (Tamura et al., 2011). The best-fit evolution model of nucleotide substitution resulted T92+G (Tamura 3-parameter + Gamma). The evolutionary history was inferred by using the Maximum Likelihood method; the bootstrap consensus tree was inferred from 500 replicates; a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 2.1279). Codon positions included were 1st+2nd+3rd. All positions con-

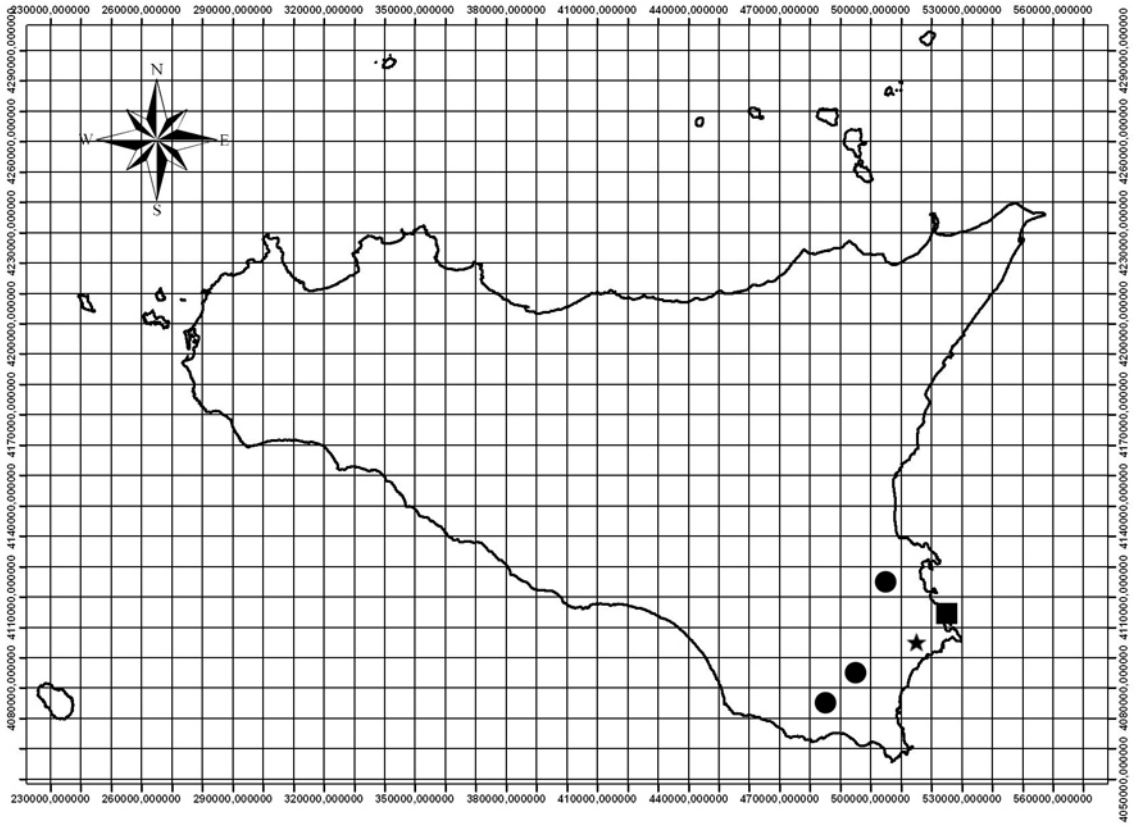


Figure 16. Geographic distribution of *Muticaria* species genetically analysed in SE Sicily: *Muticaria brancatoi* n. sp. (star), *M. syracusana* (square) and *M. neuteboomi* (dots).

taining gaps and missing data were eliminated. Divergences between SPI/SYR and SPI/NEU groups (Dxy), assessed as p distance, were 27.5% and 27%, respectively. Hence, phylogenetic tree (Fig. 15) and genetic distance between groups support the hypothesis that specimens from Spinagallo may be ascribed to a distinct *Muticaria* species.

Remarks. *Muticaria brancatoi* n. sp. appears well differentiated morphologically from nearby and strictly related species currently known. Molecular data showed a good differentiation for Spinagallo populations already in preliminary studies conducted on 16S rDNA partial sequences (Gregorini et al., 2008), but with this survey, carried out by the analysis of cytochrome oxidase subunit I gene, p distance from the other species is considerably greater.

Based on available data no evolutionary and/or paleobiogeographic hypothesis is possible, nevertheless, this work highlights a remarkable complexity (Fig. 16) and differentiation within the genus *Muticaria* in Sicily (Gregorini et al., 2008; Colomba et al., 2010), much greater than supposed until now.

ACKNOWLEDGEMENTS

We wish to thank Andrea Corso (Syracuse, Italy)

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