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ABSTRACT: Actin in *Miliammina fusca* (Brady) was identified by immunoblotting with a monoclonal antibody and compared to the actin of other foraminifera, including porcellaneous-walled Miliolina and agglutinated-walled Textulariina. *M. fusca* possesses one actin band (which molecular weight averages 46 kD), that is similar to the other Miliolina, but clearly differs from the two actin bands (at about 43 and 45 kD) observed in the other foraminifera. This result suggests a close relationship of *M. fusca* with porcellaneous foraminifera in contrast to modern taxonomy which places this species within the agglutinated foraminifera.

INTRODUCTION

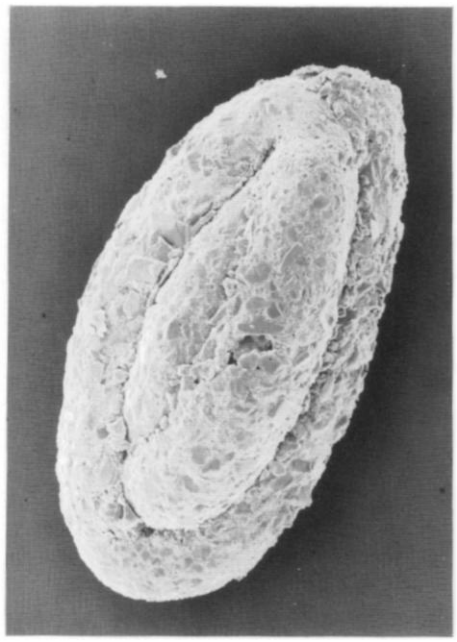
Miliammina fusca (Brady 1870) is characterized by an elongate, ovate test, with narrow chambers in typical quinqueloculine arrangement, and a rounded terminal aperture with somewhat thickened border (text-fig. 1). Its wall structure is of the Trochamminina type, with fine agglutinated particles enveloped by organic layers (Brönnimann et al. 1992). The test of *M. fusca* combines characteristics of two foraminiferal suborders, Textulariina and Miliolina (*sensu* Loeblich and Tappan 1987), in having respectively an agglutinated wall and a quinqueloculine arrangement of chambers. From an historical perspective, the taxonomic position of this species in the systematics of the foraminifera has changed depending on the importance given to the different morphological characters.

Miliammina fusca was first described by Brady (1870) and placed in the genus *Quinqueloculina* with other porcellaneous foraminifera of the family Miliolidae. This taxonomic position was followed by several authors (Schulze 1875; Cushman 1929; Blake 1933; Rhumbler 1936, etc.) for over 60 years. As far as we know, Hada (1936, 1937) was the first to transfer the species to the genus *Miliammina*. The genus *Miliammina* was first established by Heron-Allen and Earland (1930a) as a "sandy or siliceous... isomorph" of the porcellaneous genus *Miliolina* - isomorphism being defined as "the development of similar structures in unrelated organisms owing to unknown physical or biological conditions" (Heron-Allen and Earland 1930b). Heron-Allen and Earland (1930a) and Earland (1933), for example, considered the agglutinated species *Miliammina oblonga* to be isomorphic with the porcellaneous species *Miliolina oblonga, Miliammina obliqua* with *Miliolina boscianna,* and *Miliammina lata* with *Miliolina subrotunda*. These authors were firm in the belief that the similarities between *Miliammina* and *Miliolina* were not due to common ancestry, emphasizing that "even the American School of rhizopodists...would hesitate before suggesting a common ancestor for the two genera" (Heron-Allen and Earland 1930b, p. 436).

Since the genus *Miliammina* was introduced, most subsequent authors considered it as related to the agglutinated foraminifera. Heron-Allen and Earland (1930a) and Earland (1933) erected a new subfamily, the Silicininae, of the agglutinated family Lituolidae, in order to accommodate the genus *Miliammina* and other agglutinated taxa characterized by a secreted "siliceous" cement and a milioline coiling patterns. "Siliceous" as defined by Heron-Allen and Earland (1930a) and Earland (1933, p. 89), referred to tests "capable of resisting the action of strong acids without structural change." It is on this basis that *Miliammina* and other taxa were either grouped in the subfamily Rzehakininae of the family Silicinidae (Cushman 1933; 1940; 1949), or in the subfamily Rzehakininae of the family Ammodiscidae (Pokorny 1958; 1963), or in the family Rzehakinidae of the superfamily Lituolacea (Loeblich and Tappan 1964) or in the superfamily Rzehakinacea (Loeblich and Tappan 1987). Hohenegger (1990) compared phenetic and cladistic classification schemes of agglutinated and organic-walled foraminifera, concluding that the phenetic method produced better results. In both classification schemes derived from phenetic methods, *Miliammina* and the other members of the family Rzehakinidae (superfamily Lituolacea) cluster together with the superfamilies Trochamminacea and Hormosinacea which represent agglutinated foraminifera.

Over the years, only a few authors have considered *Miliammina* to be more closely related to the porcellaneous-walled Miliolina than to the agglutinated-walled Textulariina. Galloway (1933) included the genus *Miliammina* in the subfamily Miliolinae of the family Miliolidae, commenting that the "structure of this genus is too near that of other Miliolidae to belong in any other family" (p. 122) and he portrayed the genus *Quinqueloculina* as ancestral to *Miliammina*. Likewise, the genus *Miliammina* was placed in the family Paramiliolidae of the superfamily Miliolidea by Sigal (1952) and in the family Miliolidae of the superfamily Miliolacea by Haynes (1981).

In this paper, based on comparative study of actin in foraminifera, we propose to revise the taxonomic position of *Miliam-*



TEXT-FIGURE 1
Scanning electron microscopy view of the test of *Miliammina fusca* (×180).

mina fusca and we suggest to reclassify the genus *Miliammina* into the suborder Miliolina.

MATERIAL AND METHODS

Cell collection

Miliammina fusca was collected at Guilford (Connecticut, USA) in November 1994 and in the Golfe de Morbihan (Atlantic coast, France) in March 1995. Other foraminifera examined in this study were collected in different localities: *Allogromia* sp. in the Caribbean Sea (Jamaica), *Peneroplis pertusus* (Forskål) and *Triloculina rotunda* (d'Orbigny) at Ile de Porquerolles (Mediterranean Sea, France), *Amphistegina* sp. and *Textularia* sp. at Elat (Gulf of Elat, Red Sea).

Electrophoresis and immunoblotting

The foraminifera were thoroughly cleaned with a fine camel hair paint-brush before transferring into an Eppendorf test tube which contained Laemmli sample buffer supplemented with 5% 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mM p-chloromercuriphenylsulfonic acid. Cells were ground with a small plastic pestle and boiled for 3 minutes. Then, tubes were centrifuged at 10'000 g for 1 minute and supernatants were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). Gels were either silver-stained (Blum et al. 1987) or electroblotted (Towbin et al. 1979) onto nitrocellulose. The immunolabelling protocol was described elsewhere (Fahrni 1992). Anti-actin monoclonal antibody (mab) was N.350 (JLA 20, Lin 1981) from Amersham (Buckinghamshire, England). Second antibody was goat anti-mouse (GAM) IgM coupled to horseradish peroxidase (HPO), from Nordic Immunological Laboratories (Tilburg, the Netherlands). Mouse standards skeletal muscle proteins (MSK) were prepared according to Mooseker (1976) and molecular weight (MW) standards were from Bio-Rad (Richmond, CA).

RESULTS AND DISCUSSION

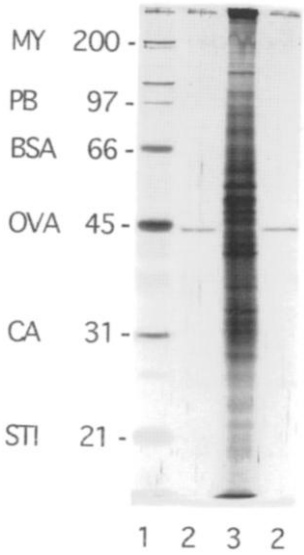
The extracts from 5 species of foraminifera were separated by SDS-PAGE, then electroblotted and immunostained with the anti-actin monoclonal antibody N.350. In the silver-stained gel, the *Miliammina* extract appears well conserved, with no evident sign of degradation in the proteins of high molecular weight (Fig. 2, lane 3). In the immuno-blot (Fig. 3), the mouse muscular actin (lanes 1 and 2, MSK) migrates as a single faint band which molecular weight (MW) is about 42 kD. Depending on the species, the foraminiferal extracts show one or two bands which do not comigrate with the mouse actin. *Peneroplis* (lane 3), *Triloculina* (lane 4) and *Miliammina* (lane 5) have a single actin band migrating at about 46 kD, *Textularia* shows one major band at about 42.5 kD and one minor band at about 45 kD (lane 6), *Allogromia* possesses two major actin bands migrating at about 43 and 45 kD (lane 7). So, the actin pattern of *Miliammina* is the same as that of the miliolids *Peneroplis* and *Triloculina*, but clearly differs from that of the agglutinated *Textularia* and the membranous-walled *Allogromia*.

The results of this study agree with those previously obtained by Fahrni and Pawlowski (1995). Three actins of different MW were detected in foraminifera: two actins of different MW are present in Allogromiina, Textulariina and Rotaliina, while only one type is present in Miliolina. The stability of the actin pattern in different taxonomic groups of foraminifera, based on the analysis of actin in about 20 species (Fahrni and Pawlowski 1995; and unpublished data) makes us confident in its use as a biochemical marker in the systematics of foraminifera.

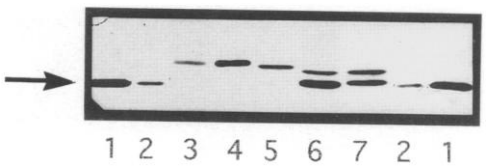
Actin suggests that *Miliammina fusca* is more closely related to porcellaneous than to agglutinated foraminifera. In consequence we propose to reclassify *Miliammina fusca* into the suborder Miliolina. This is in opposition to the generally accepted taxonomic position of the genus *Miliammina* among agglutinated taxa, in which priority is given to test wall composition (Loeblich and Tappan 1987). In the majority of the modern classification systems, the composition and structure of the test wall are regarded as the most important taxonomic characters (Haynes 1990). Moreover, their changes are viewed as major steps in evolution of foraminifera (Hansen 1979). Our data suggest that the mode of coiling may be a phylogenetically more stable character (as was recognized in some of the earliest classifications; see review in Cifelli 1990), and that the importance of the test wall characteristics has been overestimated.

This taxonomic importance contrasts with the facility with which some foraminifera may change the composition of their test wall. In particular, certain species with calcareous wall, such as *Cibicides,* can also form agglutinated, tubular extensions of their tests (Heron-Allen and Earland 1922, pl. 7, fig. 23; Nyholm 1961; Cooper 1965; Alexander and DeLaca 1987). Some estuarine miliolids, whose normal test is calcareous, may occasionally exhibit membranous specimens (Boltovskoy and Wright 1976). Other miliolids, for example *Sigmoilopsis*, are known to have a porcellaneous wall with an outer agglutinated layer (Loeblich and Tappan 1987). In other zoological groups, some demosponges are able of simultaneously secreting hydrated silica and calcium carbonate (Wood 1991).

According to our data, the priority given to the wall structure in classification of agglutinated miliolids needs to be revised. In



TEXT-FIGURE 2
SDS-PAGE analysis of a *Miliammina fusca* extract. Silver-stained 11% gel. lane 1: Molecular weight standards (MY = myosin, 200 kiloDalton; PB = phosphorylase B, 97 kD; BSA = bovine serum albumin, 66 kD; OVA = ovalbumin, 45 kD; CA = carbonic anhydrase, 31 kD; STI = soya-bean trypsin inhibitor, 21 kD); lane 2: MSK = mouse skeletal muscle standard proteins (about 0.028 µg actin); lane 3: *Miliammina* extract.



TEXT-FIGURE 3
Actin identification by immunoblotting. Part of a Western blot, in the region of the 42 kD bands (arrow), labelled with the N.350 anti-actin monoclonal antibody. Lane 1: MSK = mouse skeletal muscle standard proteins (contains about 0.070 µg actin); lane 2: MSK (contains about 0.014 µg actin); lane 3: *Peneroplis*; lane 4: *Triloculina*; lane 5: *Miliammina*; lane 6: *Textularia*; lane 7: *Allogromia.*

the case of *M. fusca*, one can speculate that this species lost the ability to secrete a calcareous test as the result of an adaptation to restricted environments characterized by low oxygen concentration and calcium deficiency. It cannot be disputed that this adaptation was successful considering the abundance of *M. fusca* in all infaunal foraminiferal assemblages, from brackish to hypersaline marshes (Murray 1991). From an evolutionary point of view, it also seems more parsimonious for a miliolid to have lost the ability to mineralize, than for a textularid to have passed through the complex series of steps involved in the development of a miliolid type of enrolment.

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REFERENCES

ALEXANDER, S. P. and DELACA, T. E., 1987. Feeding adaptations of the foraminiferan *Cibicides refulgens* living epizoically and parasitically on the Antarctic scallop *Adamussium colbecki.* Biological Bulletin, 173:136-159.

BLAKE, C. H., 1870. Order Foraminifera. In: Procter, W., Biological Survey of the Mount Desert Region, Marine Fauna. The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania. Part V:69-78.

BLUM, H., BEIER, H. and GROSS, H. J., 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis, 8:93-99.

BOLTOVSKOY, E. and WRIGHT, R., 1976. Recent Foraminifera: Dr. W. Junk b.v., Publishers, The Hague, 515 p.

BRADY, H.B.,1870. Analysis and descriptions of the foraminifera. In: Brady, G., S, and Robertson, D., The Ostracoda and Foraminifera of tidal rivers, part II. The Annals and Magazine of Natural History, ser. 4, 6:273-309.

BRÖNNIMANN, P., WHITTAKER, J.E. and ZANINETTI, L., 1992. Brackish water foraminifera from mangrove sediments of Southwestern Viti Levu, Fiji Islands, Southwest Pacific. Revue de Paléobiologie, 11:13-65.

CIFELLI, R., 1990. A history of the classification of Foraminifera (1826-1933). Part. 1. Foraminiferal classification from d'Orbigny to Galloway. Cushman Foundation for Foraminiferal Research, Special Publication, 27:1-88.

COOPER, S. C., 1965. A new morphologic variation of the foraminifer *Cibicides refulgens*. Contributions from the Cushman Foundation for Foraminiferal Research, 16 (4):137-140.

CUSHMAN, J. A., 1929. The Foraminifera of the Atlantic Ocean. Part 6. Miliolidae, Ophthalmidiidae and Fischerinidae. U.S. National Museum Bulletin, 104 (6):1-129.

CUSHMAN, J. A., 1933. Foraminifera - Their classification and economic use (2nd ed.). Special Publications Cushman Laboratory for Foraminiferal Research, 4:1-349.

CUSHMAN, J. A., 1940. Foraminifera - Their classification and economic use (3rd ed.). Harvard University Press, Cambridge, Massachusetts, 535 p.

CUSHMAN, J. A., 1948. Foraminifera - Their classification and economic use, revised and enlarged, with an illustrated key to the genera. Harvard University Press, Cambridge, Massachusetts, 605 p.

EARLAND, A., 1948. Foraminifera. Part II. South Georgia. Discovery Reports, 7:27-138.

FAHRNI, J. F.,1992. Actin in the ciliated protozoan *Climacostomum virens*: purification by Dnase I affinity chromatography, electrophoretic characterization and immunological analysis. Cell Motility and the Cytoskeleton, 22:62-71.

FAHRNI, J. F. and PAWLOWSKI, J., 1995. Identification of actins in Foraminifera: phylogenetic perspectives. European Journal of Protistology, 31:161-166.

GALLOWAY, J. J., 1933. A Manual of Foraminifera. The Principia Press Inc., Bloomington, Indiana, 483 p.

HADA, Y., 1936. Studies on the Foraminifera of brackish waters. I. Hijrippu and Mochirippu Lakes. Zoological Magazine (Japan), 48:847-860.

HADA, Y., 1937. Studies on the Foraminifera of brackish waters. II. Hachiro-Gata. III. Koyama-Ike. Zoological Magazine (Japan), 49:341-347.

HANSEN, H. J., 1979. Test structure and evolution in the Foraminifera. Lethaia, 12:173-182.

HAYNES, J. R., 1981. Foraminifera: John Wiley and Sons, New York, 433 p.

HAYNES, J. R., 1990. The classification of the foraminifera - a review of historical and philosophical perspectives. Paleontology, 33:503-528.

HERON-ALLEN, E. and EARLAND, A., 1922. Protozoa. Part II. - Foraminifera. British Antarctic ("Terra Nova") Expedition, 1910. Zoology, 6(2):25-268.

HERON-ALLEN, E. and EARLAND, A., 1930a. Some new foraminifera from the South Atlantic. III. *Mailman*, a new siliceous genus: Journal of the Royal Microscopically Society, 50 (ser. 3):38-45.

HERON-ALLEN, E. and EARLAND, A., 1930b. Siliceous shells of Protozoa [Reply to Cockerell, 1930]: Nature, 126:436.

HOHENEGGER, J., 1990. On the way to the optimal suprageneric classification of agglutinating foraminifera. In: Hemleben, C., Kaminski, M.A., Kuhnt, W. and Scott, D.B., Eds., Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera. Kluwer Academic Publishers, the Netherlands. pp. 77-104.

LAEMMLI, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

LIN, J. J. C.,1981. Monoclonal antibodies against myofibrillar components of rat skeletal muscle decorate the intermediate filaments of cultured cells. Proceedings of the National Academy of Sciences, U.S.A., 78:2335-2339.

LOEBLICH, A. R., Jr. and TAPPAN, H., 1964. Treatise On Invertebrate Paleontology. Part C. Protista 2. Sarcodina Chiefly "Thecamoebians" and Foraminiferida. The Geological Society of America and The University of Kansas Press, vol. 1-2, 900 p.

LOEBLICH, A. R., Jr. and TAPPAN, H., 1987. Foraminiferal Genera and their Classification: Van Nostrand Reinhold, New York, vol. 1-2, 970 p.

MURRAY, J., 1991, Ecology and paleoecology of benthic foraminifera. Longman Scientific & Technical, Esses, and John Wiley and Sons, Inc., New York, 397 p.

NYHOLM, K.-G., 1961. Morphogenesis and biology of the foraminifer *Cibicides lobatulus*: Zoologica Bidrag fran Uppsala, 33:157-192.

POKORNY, V., 1958. Grundzüge der Zoologischen Mikropaläontologie. VEB Deutscher Verlag der Wissenschaften, Berlin, Bd. 1.

POKORNY, V., 1963. Principles of Zoological Micropalaeontology, volume I [translation of 1958 German edition]. New York: The Macmillan Company, 652 p.

RHUMBLER, L., 1936. Foraminiferen der Kieler Bucht, gesammelt durch A. Remane, II Teil. Kieler Meeresforschungen, 1: 209-210.

SCHULZE, F. E., 1875. Rhizopodenstudien III. Archiv für Mikroskopische Anatomie, 11:94-139.

SIGAL, J., 1952. Ordre des Foraminifera. In: Piveteau, J., Traité de Paléontologie, Masson et Cie, Paris, T. I:133-178, 192-301.

TOWBIN, H., STAEHELIN, T. and GORDON, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences, U.S.A., 76:4350-4354.

WOOD, R., 1991. Non-spicular biomineralization in calcified demosponges. In: J. Reitner and H. Keupp, Eds, Fossil and Recent Sponges. Berlin and Heidelberg: Springer-Verlag, pp. 322-359.

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