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Biosystematic analysis of genus *Rattus* (Rodentia: Murinae) in Iran using total proteins of plasma and esterase-1

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Genus *Rattus* from the subfamily Murinae has a wide geographical distribution throughout the world. Three species of this genus have been reported in Iran so far. They are Norway or brown rat (*Rattus norvegicus*), black rat (*Rattus rattus*), and Himalayan rat (*Rattus pyctoris*). In order to verify the possibility of using electrophoretic patterns of special proteins, in biosystematic analysis of these species, total proteins of plasma (globulins and albumin) and esterse-1 of nine specimens belonging to three species of *Rattus*, from five populations were analyzed by native-PAGE (native polyacrylamide gel electrophoresis). Electrophoretic patterns of globulins, albumin, and esterase-1 alleles were different in these species and an unknown species was distinguished by this technique. Also, brown rats from Tehran and Mashhad were discriminated by these methods.

Key words: Iran**,** genus *Rattus*, native-PAGE*,* globulins, albumin, esterase-1

INTRODUCTION

Genus *Rattus* from the subfamily Murinae has been defined as a widely distributed and taxonomically mixed group, including many species and subspecies throughout the world (Yigit et al., 1998). They have an average body size and their tail is not completely covered with hair (Etemad, 1975). Three species of this genus have been reported in Iran so far. One of them is the brown rat, *Rattus norvegicus*, which was originally found in the southern coasts of Caspian Sea. This species has been transported to Tehran, Mashhad, Shiraz, Tabriz and some other places (Etemad, 1975). Their tail length is always less than the head and body length in adult specimens and sometimes it is equal to the head and body length in young specimens (Yigit et al., 1998). Ear is short and when drawn forward, does not reach the eyes (Etemad, 1975). The dorsal part of the body is covered with coarse, brownish fur which usually lightens to a grey or tan color near the underside. Temporal ridges on the brain case are straight and almost parallel (Darvish et al., 2006). The incisiva foramen has a moderate size, just reaching the front of M1 (Yigit et al., 1998).

Another species is the black rat, *Rattus rattus*, which is native to Indian Peninsula and was introduced worldwide in the temperate zone and parts of the tropical and subantarctic zones (Musser and Carleton, 2005). It has been distributed to the border of Persian Gulf from the south east by ships. Currently, this species lives either simbiotically with human or partially independently in places such as mangrove forests and Qeshm Island (Etemad, 1975). Their tail always exceeds the head and body in length. When the ear is drawn forward, it reaches the eyes and usually exceeds

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them. The general features of the skull are the same as those of *R. norvegicus*, but parietal and the interparietal bones are bordered by ridges which are almost convex. The incisiva foramen is relatively longer than *R. norvegicus* (Yigit et al., 1998).

The Himalayan Rat or *R. pyctoris* is the third species, which is distributed in the south east of Kazakhstan, Kyrgyzstan, east of Uzbekistan and Tajikistan, north of Afghanistan, north of Pakistan, north of India, Nepal, south of China and north east of Iran, such as the mountains in the vicinity of Mashhad, Zoshk village, Jaghargh, Noghondar and Golmakan in the south of Chenaran. This species is also reported from north east of Kerman province of Iran (Musser and Carleton, 2005). Its upper parts are light brown with shading of grey. Bases of hair are slate grey and the tips are russet or yellowish-tinged.**.** The parietal region is a little inflated and crests of frontals at the margin of orbits are fused with the parietal crests without interval (Darvish et al., 2006).

Biochemical population genetic techniques are used for examining genetic variation and species genetic isolation at the level of species. Electrophoresis is the most widely used technique for biochemical systematics. Electrophoretic mobility is used as an indicator of similarity of amino acid composition when orthologous proteins are compared between individuals. The character examined is the mobility of a protein under a given set of electrophoretic conditions. A difficulty which arises in the use of non-specific general protein is the problem of homologus bands of equivalent position on the gel in samples from different species which may not necessarily represent orthologous proteins. Therefore, for most systematic analyses, staining for specific enzymes such as esterase-1 is preferred and reduces the problems of homology (Ferguson, 1980). Electrophoretic aspects of blood serum proteins and esterases of genus *Ratttus*, specially *R. norvegicus* and *R. rattus* have been studied in Turkey (Verimli et al., 2000; Yigit et al., 2001), but no study has been carried out to verify the variation of these species captured in different locations in Iran. This prelimonay study aims to assess whether the electrophoretic aspects of total proteins and esterase-1 of plasma could have a taxonomic value for discrimination of the different species of genus *Rattus* in Iran.

MATERIAL AND METHODS

The electrophoretic analysis was performed on nine live specimens collected from five locations using live traps in the distribution areas of *R. norvegicus*, *R. rattus*, and *R. pyctoris* in Iran (Fig. 1). 2 (FUMZM1453, FUMZM 1655) *R. norvegicus* from Mashhad (36˚ 17´N/59° 36´E), 3 (FUMZM 1628, FUMZM 1629, FUMZM 1630) *R. norvegicus* from Tehran, Rey (35° 34´N/51° 26´E), 2 (FUMZM 1663, FUMZM 1674) *R. rattus* from Shiraz (29° 36´N/52° 32´E) and mangrove forests of Tiab of Minab (27° 07´ 04˝N/56° 53´ 41˝E), 2 (FUMZM 1659, FUMZM 1660) *R. pyctoris* from Noghondar village (36° 22´ 23˝N/59° 17´ 35˝E), and also an unknown species (1654) from Semnan (35° 34´N/53° 23´E)-Mashhad train.

For electrophoresis of total proteins and esterse-1 of plasma by native-PAGE (native polyacrylamide gel electrophoresis), the blood was taken by cardiac puncture from the animals, which had been anaesthetized with ether. In order to prevent blood coagulation, blood samples were mixed with 5% w/v EDTA in physiological saline (0.85% w/v NaCl in deionized water). Then, for separating the plasma, blood samples were centrifuged at 3600 rpm for 10 min. and supernatants were stored at -70ºC before electrophoresis. Bradford assay was used to determine the proteins in plasma, and their absorbance was measured using NanoDrop (ND-1000). Then, 25 µg (for total proteins assessment) or 50 µg (for esterase-1 assessment) of proteins in plasma were mixed with equal volumes of a 1X sample buffer, containing 0.1% bromophenol blue and 10% glycerol in deionized water (Sambrook et al., 1989). Electrophoresis was carried out using a vertical slab gel electrophoresis apparatus (WEU-7305D). 12% native polyacrylamide gels were prepared as described by Britton-Davidian

FIGURE 1. Sampling localities Map in this study. *R. rattus* (1, 2), *R. norvegicus* (3, 4), *R. pyctoris* (5), *R.* sp. (6).

(1993). The electrode buffer solution used was Lithium hydroxide as described by Selander et al. (1969 and 1971). 25 mg/ml of BSA in deionized water (Bovine Serum Albumin, Sigma) with molecular weight of 66430 Dalton was used as a marker for electrophoresis on the native gel (Ferguson, 1980) and a constant voltage (12 V/cm) was applied to the gels.

For analysis of total proteins, the gels were stained with the silver nitrate solution as described by Sambrook, et al. (1989), and in order to visualize esterase-1 bands, they were stained with 4ml sodium phosphate buffer containing 1:1 mixture of 0.1 M monobasic sodium phosphate, pH 4.4 and 0.1 M dibasic sodium phosphate, pH 8.7, 1ml 1% NADA (Naphthol-As-D Acetate or 3-(Acetyloxy)-*N*-(2-methylphenyl)-2-naphthalenecarboxamide**,** Sigma) in acetone as a substrate, 25 mg fast blue RR salt (Sigma) and 45 ml water. These gels were incubated in this solution for 10-30 min at 37°C (Selander et al., 1971; Van Deusen and Kaufman, 1978). Then, all gels were scanned and their electrophoretic pattern was drawn with TotalLab v. 1.10 and Photoshop softwares. Then, Neighbor-Joining dendrograms were constructed and analyzed by PAUP v. 4.0b10.

RESULTS AND DISCUSSION

After electrophoresis of blood plasma proteins of rats and gells were stained with silver nitrate solution and two different regions were observed on the gel. After comparing BSA marker with 66

kDa molecular weight, globulins and albumin regions were determined. As shown in Figure 2, after electrophoresis, each three species of *Rattus* showed different banding patterns (Fig. 2).

Also, using BSA marker, the position of esterase-1 on the gel was determined. After staining with NADA as a substrate, a single esterase locus, migrating to the anode in a region close to 66 KDa (Fig. 3) was distinguished. In different species, this locus has two alleles: a fast allele (Es-1a) and a slow one (Es-1b), and they are autozomal locuses (Womack, 1973). Homozygote species (*R. norvegicus* from Tehran and *R. pyctoris*) have one of these alleles and heterozygote species (*R. norvegicus* from Mashhad and 2 *R. rattus*) have two alleles. According to Womack (1973) esterase-1 in serum of rats has two alleles (Es-1a and Es-1b) and there is no difference between serum and plasma proteins.

These specimens of *Rattus* were analyzed by Neighbor-Joining program of PAUP, based on banding patterns on electrophoretic gels (Fig. 4). To do so, first, the bands of proteins and each allele of esterase-1 were numbered according to their movement on the gel. The fastest band was named 100 and then 95, 90 and so on. Then, they were shown with 0 and 1 characters for different specimens (Table 1).

Esterase enzymes are considerably polymorphic, and therefore, these loci are not suitable for separating taxa (Matsumoto et al., 1979) and according to Verimli, et al. (2000), there is also very poor information on non-specific esterases of wild populations in *Rattus norvegicus*. In this study, esterase-1 enzyme in plasma with two alleles (Womack, 1973) was detected using NADA substrate and it could separate three species of *Rattus*. NADA was found to be a good substrate for detecting esterase-1 in plasma in these species. Van Deusen and Kaufman (1978) also used NADA substrate for detection of esterase-1 in liver of *Promyscus*, and found it was suitable for assessment of esterase-1 present in liver and hemolysate. On the other hand, Selander & Yang (1969) and Selander, et al. (1971) used α-naphthyl propionate as a substrate for liver and kidney Es-1 of *Promyscus polinotus* and *Mus musculus*.

Matsumoto et al. (1979) showed that age and sex can influence the pattern of serum esterase-1 in laboratory rats, when using α-naphthyl butyrate as a substrate, but in this study, using NADA as a substrate, showed that sex did not have any effect on esterase-1 of plasma in wild rats (Fig. 3). Also according to Reuter and Kennes (1966) on the basis of the horizontal starch gel electrophoresis techniques, the electrophoretic pattern of females *Mus* was significantly different from that of the males with respect to the prealbumin zone. On the other hand, blood serum proteins of *Rattus* in Turkey, using SDS-PAGE, revealed that there was no difference between sexes (Yigit et al., 2001). In this study, using 12% native-PAGE for analyzing total proteins of plasma, no difference was observed between sexes (Fig. 2).

Yigit et al. (2001) reported that globulins, albumin, post albumin, and pre albumin have different electrophoretic patterns in populations of *R. rattus* and *R. norvegicus* in Turkey when using SDS-PAGE to separate blood serum proteins. Despite the variations in the electrophoretic patterns of both species, these patterns seem to be inapplicable as diagnostic characters in distinguishing *R. rattus* from *R. norvegicus*. But, in this study, using native-PAGE and drawing dendrograms for globulins, albumin, and esterase-1 were helpful for distinguishing all three species of *Rattus* in Iran, *R. norvegicus*, *R. rattus*, and *R. pyctoris*, and also for identifying an unknown species.

Three species of *Rattus* in Iran, *R. norvegicus, R. rattus,* and *R. pyctoris*, were distinguishable with electrophoretic patterns and Neighbor-Joining dendrograms of total proteins and esterase-1 of plasma. In addition an unknown species from Semnan was found to be close to *R. norvegicus*.

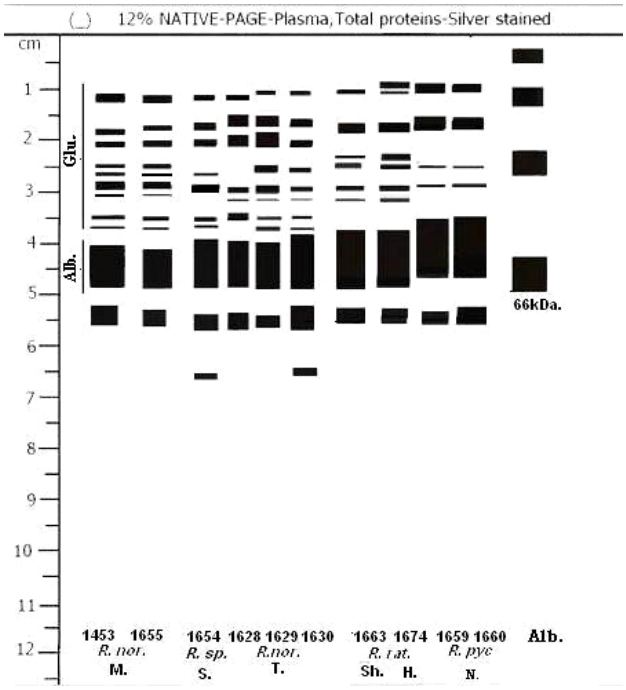

FIGURE 2. Native-PAGE patterns of plasma proteins of *Rattus*. *R. norvegicus* from Mashhad (FUMZM 1453, FUMZM 1655), *R. norvegicus* from Tehran (FUMZM 1628, FUMZM 1629, FUMZM 1630), *Rattus* sp. from Semnan (FUMZM1654), *R. rattus* from Shiraz (FUMZM1663), *R. rattus* from mangrove forests (FUMZM 1674), *R. pyctoris* from Noghondar (FUMZM 1659, FUMZM 1660), and Alb marker. ♂: male, ♀: female.

TABLE 1- Numeric values of electrophoretic bands of total proteins and esterase-1 of plasma.

	Species	1453 *R.nor.* M.	1655 *R.nor.* M.	1654 *R.sp.* S.	1628 *R.nor.* T.	1629 *R.nor.* T.	1630 *R.nor.* T.	1663 *R.rat.* Sh.	1674 *R.rat.* Mang.	1659 *R.pyc.* N.	1660 *R.pyc.* N.
	Bands										
Total proteins	100	1	1	1	1	1	1	1	1	1	1
	95	1	1	1	1	1	1	1	1	0	0
	90	0	0	0	0	0	0	0	0	1	1
	85	1	1	0	0	1	1	0	0	0	0
	80	0	0	1	0	0	0	0	0	0	0
	75	1	1	1	1	1	1	0	0	0	0
	70	0	0	0	1	1	1	1	1	0	0
	65	1	1	0	0	0	0	0	0	0	0
	60	0	0	1	1	1	1	1	1	0	0
	55	1	1	0	0	0	0	0	0	1	1
	50	1	1	1	0	1	1	0	0	0	0
	45	1	1	0	0	0	0	1	1	1	1
	40	0	0	0	0	0	0	1	1	0	0
	35	1	1	1	1	1	1	0	0	0	0
	30	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	0	0	0	0	0	0
	20	0	0	0	0	1	1	1	1	1	1
	15	0	0	0	0	0	0	0	1	0	0
Esterase-Es-	100	1	1	0	0	0	0	0	0	0	0
	95	0	0	0	0	0	0	1	1	0	0
	90	0	0	1	1	1	1	0	0	0	0
Esterase-Es-	100	0	0	0	0	0	0	1	1	0	0
	95	1	1	0	0	0	0	0	0	0	0
	90	0	0	0	0	0	0	0	0	1	1

Numeric values of electrophoretic bands detected for total proteins and esterase-1 of plasma in 10 specimens of *Rattus* from Iran. *R. norvegicus* of Mashhad (FUMZM 1453, FUMZM 1655), *Rattus* sp.(FUMZM 1654), *R. norvegicus* of Tehran (FUMZM 1628, FUMZM 1629, FUMZM 1630), *R. rattus* of Shiraz (FUMZM 1663), *R. rattus* of Harra forests (FUMZM 1674), and (FUMZM 1659, FUMZM 1660) *R. pyctoris* of Noghondar.

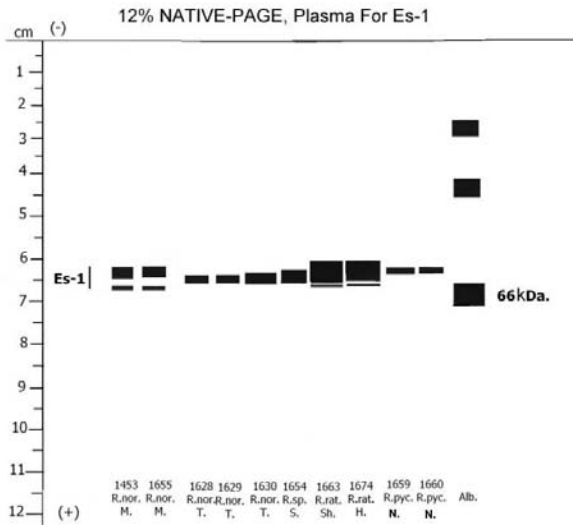

FIGURE 3. Zymogram of the gel electrophoresis for esterase-1 of plasma using NADA as a substrate. *R. norvegicus* from Mashhad (FUMZM 1453, FUMZM 1655), *R. norvegicus* from Tehran (FUMZM 1628, FUMZM 1629, FUMZM 1630), *Rattus* sp. from Semnan (FUMZM 1654), *R. rattus* from Shiraz (FUMZM 1663), *R. rattus* from mangrove forests (FUMZM 1674), *R. pyctoris* from Noghodar (FUMZM 1659, FUMZM 1660), and Albumin marker. ♂: male, ♀: female.

It was noteworthy that, using this technique, *R. norvegicus* from Mashhad and Tehran were separated from the dendrograms, which were drawn based on the electrophoretic patterns of both total proteins of plasma and esterase-1. This shows that most probably, *R. norvegicus* from Mashhad have different origin from Tehran population.

As seen in Figure 4, in the dendrogram *R. rattus* specimens from mangrove forests and Shiraz are placed close together based on both their total proteins and esterase-1.

It is also found that *R. rattus* and *R. pyctoris* are more similar together when compared to *R. norvegicus*. This similarity was also true for their morphological characters (data not shown). This is different from Musser and Carleton's classification (2005), in which *R. pyctoris* is placed in group of *R. norvegicus*. Furthermore, using 12% native-PAGE for analysing total proteins and esterase-1 of plasma, revealed no difference between sexes.

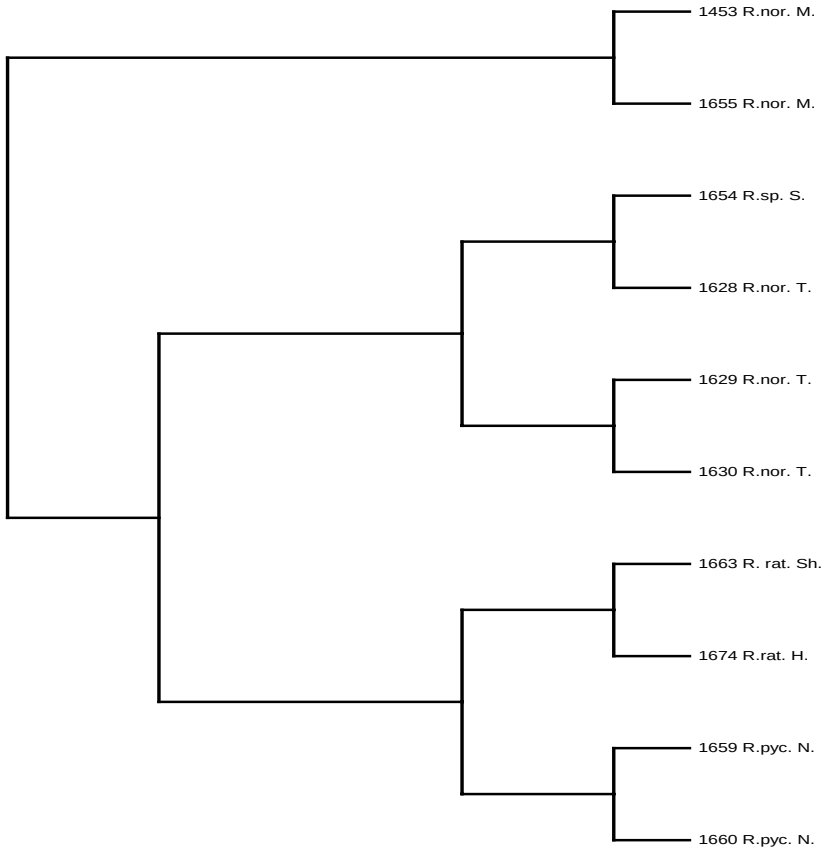

FIGURE 4. Neighbor-Joining dendrogram showing distances between *Rattus* species after analysing total proteins of plasma and esterase-1 activity. *R. norvegicus* from Mashhad (1453, 1655), *Rattus* sp. from Semnan (1654), *R. norvegicus* from Tehran (1630, 1629, 1628), *R. rattus* from Harra forests (1674), *R. rattus* from Shiraz (1663), and *R. pyctoris* from Noghondar (1660, 1659).

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LITERATURE CITED

Britton-Davidain, J., 1993. Starch gel electrophoresis in vertebrates. *Methods in Enzymology* 224, 98-112.

Darvish, J., Siahsarvie, R., Mirshamsi, O., Kayvanfar, N., Hashemi, N., Shakib, F.S., 2006. Diversity of the rodents of northeastern Iran. *Iranian Journal of Animal Biosystematic* 2(1), 54-76.

Etemad, E., 1975. *Mammals of Iran (Rodantia*), 3rd edition. National society of protection of natural sources and human environment publications, Tehran.

Ferguson, A., 1980. Biochemical systematic and evolution. Wiley Publisher, New York.

Matsumoto, K., Syuto, B., Miyake, Y.I., Matsuhashi, A., Aizawa, M., 1979. Serum esterase genetics: Identification and hormone induction of the Es-1b esterase in inbred Rats, *Biochemical genetics* 79 (11/12), 1121-1129.

Musser, G.G., Carleton, M.D., 2005. Superfamily Muroidea. In: Wilson D.E., Reeder, D.M. (Eds.). *Mammal species of the world, a taxonomic and geographic reference*. 3rd edition. Johns Hopkins University Press, Baltimore, OR pp. 894-1494.

Reuter, A., Kennes, F., 1966. Strain and sex dependency of pre-albumin in mice. *Nature* 210,745.

Sambrook, J., Fritch, E.F., Maniatis, T., 1989. *Molecular cloning, a laboratory manual*, 2nd edition. Cold Spring Harbor Laboratory Press, New York.

Selander RK, Smith MH, Yang SY, Johnson WE, and Gentry JB, 1971. Biochemical polymorphism and systematics in the genus *Peromyscus* I. Variation in the old field mouse (*Peromyscus polionotus*). University of Texas publication 7103. Austin: University of Texas; 49–90.

Selander, R.K., Yang, S.Y., 1969. Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*). *Genetics* 63, 653-667.

Van Deusen, M., Kaufman, D.W., 1978. Esterase-1 as a genetic marker for *Peromyscus*, *Journal of Mammology* 59(10), 158-186

Verimli, R., Yigit, N., Colak, E., Sözen, M., 2000. Nonspecific esterase patterns of *Rattus norvegicus* (Berkenhout, 1769) in western Turkey, *Turkish Journal of Biology* 24, 825-831

Womack, J.E., 1973. Biochemical genetics of rat esterases: polymorphism, tissue expression, and linkage of four loci. *Biochemical Genetic* 9(1), 13-24.

Yigit, N., Colak, E., Cözen, H., Özkurt, S., 1998. The taxonomy and karyology of *Rattus norvegicus* (Berkenhout, 1769) and *Rattus rattus* (Linnaeus, 1758) (Rodentia: Muridae) in Turkey. *Turkish Journal of Zoology* 22, 203-212

Yigit, N., Verimli, R., Colak, E., Sözen, M., Özkurt, S., 2001. Blood-serum proteins of *Rattus rattus* and *Rattus norvegicus* (Mammalia: Rodentia) in Turkey. *Turkish Journal of Biology* 25, 83-88.