



# Do Seismic Signals Diverge at the Level of Cytotypes in Turkish Blind Mole Rats?

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## ABSTRACT

Subterranean animals are limited in mobility and have reduced sensory ability due to living underground, and therefore are presented with a challenge to communicate. One solution is to use seismic signalling, by head drumming, to convey species-specific information. The lesser blind mole rats (*Nannospalax* sp.) are obligate subterranean rodents known for their remarkable chromosomal variation. In the present study, we investigated whether the structure of seismic signalling is different between the two species found in Turkey, *Nannospalax leucodon* and the *N. xanthodon* and whether it is associated with ecological, sex, temporal and chromosomal peculiarities. A cytotype of *N. leucodon* ( $2n=56$ ) and three cytotypes of *N. xanthodon*, ( $2n=38$ ,  $52$  and  $60$ ) were used in the study. We observed no seismic signalling in *N. leucodon*. In *N. xanthodon*, the cytotype  $2n=60$  had faster rate of signalling in comparison to two other cytotypes ( $2n=38$  and  $2n=52$ ), and the cytotypes also differed significantly in the structural components of their signals. There was no difference in signal pattern between different fundamental number variations within cytotypes. We observed temporal variation in seismic signals, but did not find any difference in signalling between the sexes, suggesting the signals are not used for mate recognition. In addition, the signalling structure was not associated with the climate and the soil types of the habitat of origin. We suggest that seismic communication by drumming may be used to recognize conspecifics within the same cytotype or species.

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## Authors' Contribution

FÇ and FM collected samples together in the field and designed the study. FÇ made the behavioural experiments, analysed the data and prepared draft. FM edited the manuscript.

## Key words

Head drumming, Vibrational communication, *Nannospalax xanthodon*, *N. leucodon*, Behavioural isolation.

## INTRODUCTION

Reliable means of communication are essential between conspecifics or different taxonomic groups in practically all animals. Surface-dwelling species may communicate via a number of modes, including visual, audio and chemical; however, none of these are suitable for long-distance communication underground. Therefore, the means of communication in subterranean species is substantially adapted for living underground (Burda *et al.*, 2007; Lange *et al.*, 2007; Heth *et al.*, 1985, 2002; Kimchi and Terkel, 2002; Heffner and Heffner, 1992, 1993). It is impressive that other sensory channels and organs can compensate for the lack of usual communication means (Nevo, 1990). Seismic signals, in particular, are produced in several ways in rodents: by the hind feet, as in Bathyergidae and Dipodidae (Mason and Narins, 2001), both the hind feet and incisors, as in Muridae (Giannoni *et al.*, 1997; Bennett and Jarvis, 1988;

Randall and Stevens, 1987; Ward and Randall, 1987, Dewsbury *et al.*, 1978; Howe, 1978; Kenagy, 1973, 1976; Eisenberg, 1963), and by the head drumming, as in Spalacidae (Hrouzková *et al.*, 2013; Li *et al.*, 2001; Heth *et al.*, 1987; Rado *et al.*, 1987). We used the seismic signal term as the vibrations which animal produced it by hitting of a body parts to a substrate such as to the ground (Giannoni *et al.*, 1997; Hrouzková *et al.*, 2013; Rado *et al.*, 1987; Randall, 199; Heth *et al.*, 1987). Blind mole rats (Spalacidae) use seismic signalling to advertise their territory, as well as for orientation (Kimchi *et al.*, 2005; Kimchi and Terkel, 2002, 2003; Heth *et al.*, 1987, 1991), and possibly for species identification (Heth *et al.*, 1987).

If seismic signalling is used for conspecific communication, it should differ between reproductively isolated species. While *Dipodomys* also produce species-specific drumming (Randall, 1997), head drumming signals were found to be specific to cytotypes in the Palestinian blind mole rat *Nannospalax ehrenbergi* in Israel (Heth *et al.*, 1991; Nevo, 1985; Nevo *et al.*, 1987; Rado *et al.*, 1987).

Differences in communication signals among populations might depend on different ecological conditions (Seehausen *et al.*, 1997; Marchetti, 1993), but

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can also serve as a mechanism of behavioural isolation (Boul *et al.*, 2007; Seehausen *et al.*, 1997). The differences in head drumming signalling were suggested to contribute to behavioural isolation and speciation among the different populations of the Palestinian BMR (*N. ehrenbergi*) in Israel (Nevo *et al.*, 1987).

In the Turkish fauna *N. xanthodon* is one of the three *Nannospalax* species, represented by more than 30 cytotypes. It is widely distributed in Turkey (Arslan *et al.*, 2010, 2016; Arslan and Zima, 2014; Kankilic and Gürpınar, 2014; Matur *et al.*, 2013; Hadid *et al.*, 2012; Kandemir *et al.*, 2012; Kryštufek *et al.*, 2012; Kankılıç *et al.*, 2010; Kryštufek and Vohrálik 2009; Yiğit *et al.*, 2006; Sözen *et al.*, 2006; Matur and Sözen, 2005; Sözen, 2004). *N. leucodon* is also represented by multiple cytotypes, but only one ( $2n=56$ ) is found in Turkey's Thrace (Sözen *et al.*, 2006). Notably, the blind mole rats in Turkey occupy a wide range of habitats (e.g. different climate and soil types, Eken *et al.*, 2005; Atalay, 2004).

Aside from a few reports on seismic signalling behaviour in *N. ehrenbergi* (Nevo *et al.*, 1987; Rado *et al.*, 1987), there has been no investigation on the other species of mole rats (Hrouzková *et al.*, 2013; Li *et al.*, 2001). We aimed to extend our knowledge on the head

drumming signalling of two blind mole rats species found in Turkey (*N. leucodon* and *N. xanthodon*) in respect to their chromosomal and ecological characteristics.

## MATERIALS AND METHODS

### Sampling

We examined 22 adult individuals of *N. leucodon* from 2 locations and 135 individuals of *N. xanthodon* from 10 locations in Turkey, characterized by different habitats. The individuals of *N. xanthodon* belonged to three distinct cytotypes (Table I; Fig. 1). Karyotype of individual were determined by Matur *et al.* (2005) and (2013). In this study we collect identical coordinates with those already published so we didn't repeat the karyotype.

### Housing

Animals captured in the field were transferred individually to the laboratory in nest cages ( $40 \times 25 \times 20$  cm). A plastic tunnel protruded from one side of the cage to simulate a natural environment (underground tunnels) for the animals. Sawdust was added to the cages as burrowing material and bedding and the animals were fed carrots, lettuce, celery, apples, wheat, corn and cabbage *ad libitum*.

**Table I.- Sampling localities of *N. xanthodont* (1 to 10<sup>th</sup> localities) and *N. leucodon* (11 and 12<sup>th</sup> localities). Also the race name described by Arslan *et al.* (2016) were given in brackets under the locality names.**

| Locality number | Locality              | 2n              | FN | n  |     | No. of individuals displaying seismic activity |         | Climate type*         | Soil type         | Coordinates              |
|-----------------|-----------------------|-----------------|----|----|-----|------------------------------------------------|---------|-----------------------|-------------------|--------------------------|
|                 |                       |                 |    | F  | M   | Presence                                       | Absence |                       |                   |                          |
| 1               | Karacabey (turcicus)  | 38              | 74 | 2  | 3   | -                                              | 5       | S. humid              | Clay              | 40°14.844'N; 28°15.212'E |
| 2               | Bigadiç (turcicus)    |                 |    | 13 | 6   | 10                                             | 9       | S. arid               | Clay              | 39°22.203'N; 27°59.543'E |
| 3               | Çanakkale (turcicus)  |                 |    | 8  | 4   | 4                                              | 8       | S. arid               | Clay              | 40°10.349'N; 26°29.054'E |
| 4               | Yalova (abant)        | 52 <sup>a</sup> | 74 | 2  | 3   | 5                                              | 0       | Humid                 | Clay              | 40°37.229'N; 29°20.574'E |
| 5               | Gerede (abant)        |                 | 72 | 17 | 11  | 6                                              | 22      | S. humid              | Clay              | 40°47.631'N; 32°9.387'E  |
| 6               | Korkuteli (vasvarii)  | 60 <sup>a</sup> | 74 | 4  | 2   | 5                                              | 1       | S. arid               | Clay              | 37°13.359'N; 30°16.253'E |
| 7               | Kapaklı (vasvarii)    |                 | 78 | 13 | 14  | 15                                             | 12      | S. humid              | Sand <sup>d</sup> | 40°50.033'N; 32°26.724'E |
| 8               | Oklubal (vasvarii)    |                 |    | 10 | 6   | 10                                             | 6       | S. arid               | Clay <sup>d</sup> | 39°48.951'N; 30°14.108'E |
| 9               | Gölbaşı (vasvarii)    |                 | 80 | 4  | 5   | 6                                              | 3       | Arid <sup>c</sup>     | Clay              | 39°42.057'N; 32°49.563'E |
| 10              | Tavşanlı (vasvarii)   |                 |    | 4  | 5   | 9                                              | -       | S. humid <sup>c</sup> | Clay              | 39°35.161'N; 29°24.378'E |
| Sub total       |                       |                 |    | 77 | 59  | 67                                             | 69      |                       |                   |                          |
| 11              | Babaeski (leucodon)   | 56              | 78 | 6  | 8   | -                                              | 14      | S. humid              | Clay              | 41°29.212'N; 26°57.585'E |
| 12              | Lüleburgaz (leucodon) |                 |    | 3  | 5   | -                                              | 8       | S. humid              | Clay              | 41°21.356'N; 27°33.278'E |
| Sub total       |                       |                 |    | 9  | 13  |                                                |         |                       |                   |                          |
| Total           |                       |                 |    |    | 158 |                                                |         |                       |                   |                          |

\*S. Humid, semi-humid, <sup>a</sup>Cytotypes where comparison has been made among the fundamental chromosomal arms (FN). <sup>b</sup>Number of individual used for repeated measurements. <sup>c</sup>Localities where comparison has been made among the climate types. <sup>d</sup>Localities where comparison has been made among the soil types.

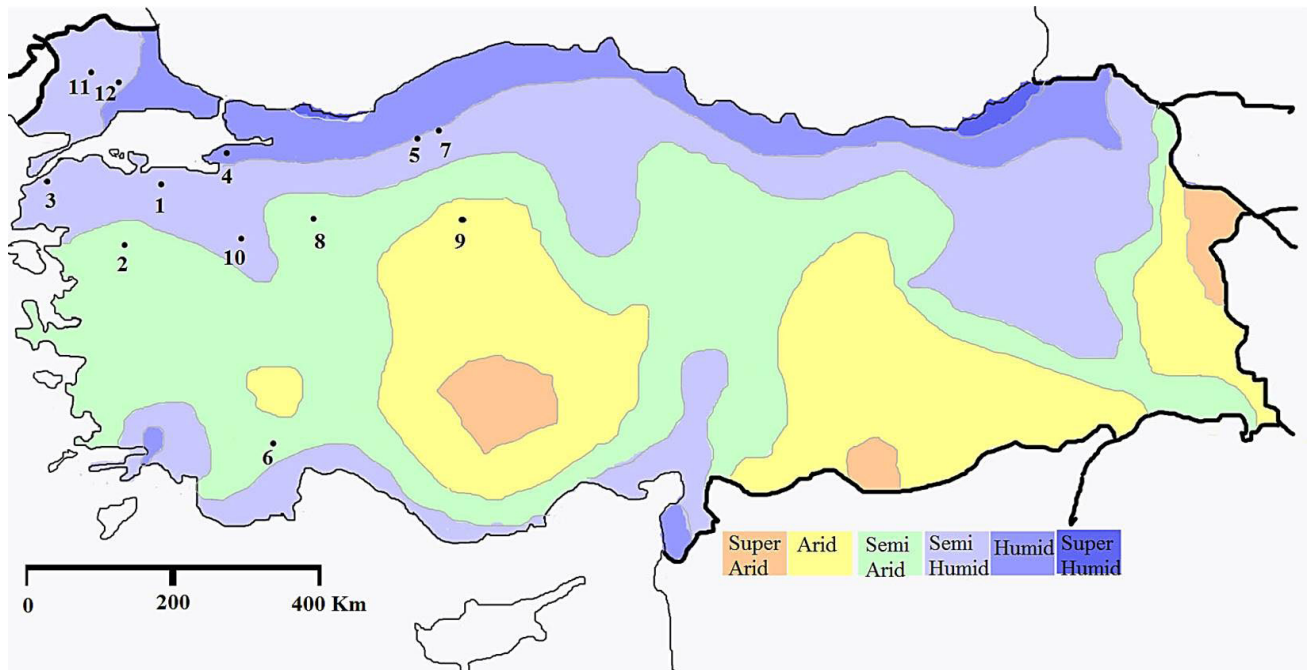


Fig. 1. Climate types of sampling localities of *N. xanthodon* and *N. leucodon* (Eken *et al.*, 2005). The cytotype and locality information are given at Table 1.

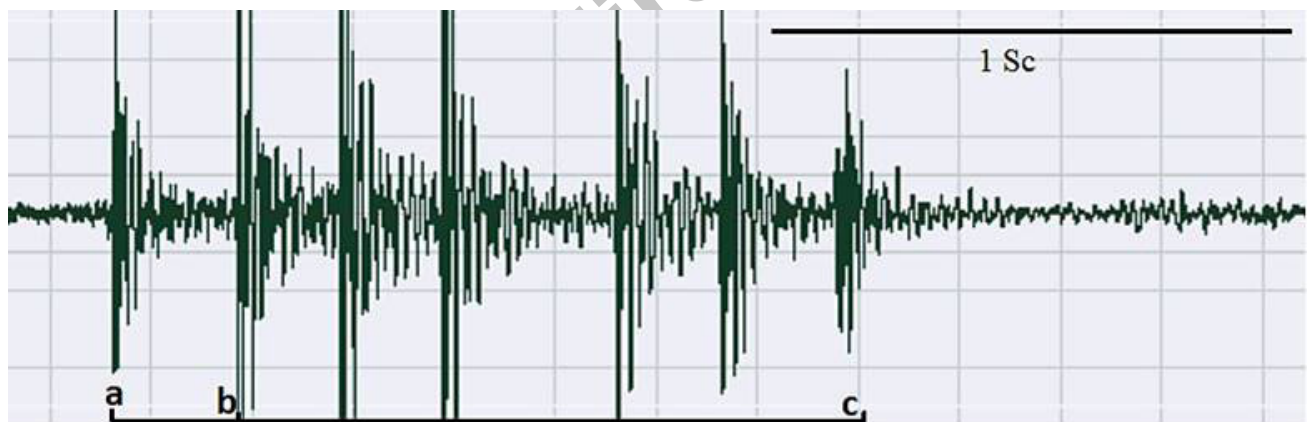


Fig. 2. Sonogram of a signal and parameters measured. PGS, pulse group size or width of a signal (elapsed time between the points of a–c; PG, pulse group in a burst, peak count between the points of a–c; and PU or signal speed, the one pulse or thump of animals in one burst, distance between two peaks, elapsed time between the points of a–b; Upper bar shows 1 sec (second).

### Procedure

We performed all behavioural experiments in the laboratory, taking into account the previous findings by Rado *et al.* (1987), who showed that seismic signalling patterns in *N. ehrenbergi* did not differ between the field and the laboratory conditions. Animals were allowed a familiarization period of one week before the experiments began (Zuri and Terkel, 1996). As in kangaroo rats (*Dipodomys*), the drumming rates are expected to increase

when animals are stressed (Randall and Matocq, 1997; Randall, 1991); therefore, we paid maximum attention to minimize the stress level. In earlier studies (Heth *et al.*, 1987; Rado *et al.*, 1987), the drumming behaviour was initiated by introducing two animals into the same tunnel, but other methods that avoid direct contact between animals are now preferred to avoid unnecessary injury. For that reason, we only allowed a single individual into the tunnel. The signals by each animal were recorded in its

own environment and no other animal was used to prompt the drumming behaviour. A soft material made of sponge or Styrofoam was rubbed on the plastic tunnel to provoke drumming. This way, if the animal started drumming to mark its territory it did not have to defend itself against another animal. The signalling pattern was recorded by placing an audio recording device (SONY IC Recorder ICD-PX330 with integrated microphone) against the sidewall of the plastic tunnel. The recording was repeated two or three times per week and the entire experiment was completed within two months (15 repetitions). At the end of the experiment, the animals were euthanized (Leary *et al.*, 2013), as it was not possible to return them back to their natural environment. Subsequently, we calculated the signal parameters with an audio analysis program Adobe Audition 3.0 (Riley, 2008) as described by Hrouzková *et al.* (2013), Rado *et al.* (1987), Randall (1997) and Heth *et al.* (1987). The calculated parameters from a signal set were as follows: PGS, pulse group size or width of a signal (elapsed time between the points of a–c; Fig. 2); PG, pulse group in a burst (peak count between the points of a–c; Fig. 2); and PU or signal speed, the one pulse or thump of animals in one burst, distance between two peaks (elapsed time between the points of a–b; Fig. 2). The signal or burst refers to the elapsed time between the points of a–c and consists of the time between starting and ending points of the drumming of the blind mole rats as shown on Figure 2.

#### Statistical analysis

We first tested whether the differences in seismic signalling among individuals were not random by using the signals recorded during different sessions on different days. First, we applied ANOVA (SPSS 13) with repeated measurements for each animal for the PU parameter and a non-parametric Friedman test for the PGS and PG parameters (SPSS 13). Second, we tested if signal components varied by sex by using the t-test on the data for three different variables (PU, PGS, and PG). To test whether ecological characteristics have any effect on the seismic signal patterns, we performed the same testing within cytotypes. To test a single ecological variable per each step, we pooled together individuals with identical FN values from different localities. We used a total of 27 individuals from Kapaklı ( $2n=60$ ,  $FN=78$ , sandy soil type, locality no= 7 in Fig. 1), and 16 animals from Oklupal ( $2n=60$ ,  $FN=78$ , clayish soil type, locality no = 8 on Fig. 1; Table I). We tested for the effects of climate by using a total of 9 individual from Gölbaşı ( $2n=60$ ,  $FN=80$ , arid climate, locality no = 9 in Fig. 1), and 9 individuals from Tavşanlı ( $2n=60$ ,  $FN=80$ , semi-arid climate, locality no = 10 on Fig. 1). Finally, we analyzed the variation in seismic signalling among all FN types within two

cytotypes ( $2n=52$ :  $FN=72$ , 74 by the t-test and  $2n=60$ :  $FN=74$ , 78, 80 by ANOVA) and among all cytotypes of *N. xanthodon* ( $2n=38$ , 52 and 60) by ANOVA. The PCA was performed in R statistical software, using all three variables, to see whether the clustering pattern in individual data corresponded to cytotypes or to FN types in two-dimensional space (Kassambara, 2016; Kassambara and Mundt, 2016; R Development Core Team, 2016).

The animals were treated in accordance with the guidelines of the Bülent Ecevit University Animal Experiments Local Ethic Committee; approval reference number 2012-03-25/01.

**Table II.- Cytotypes comparison (ANOVA shows the results of three characters among cytotype characters. f: F value).**

| Variable | 2n | n  | Mean  | Test value           | p-value |
|----------|----|----|-------|----------------------|---------|
| PU       | 38 | 10 | 0.115 | 103.499 <sup>f</sup> | 0.000*  |
|          | 52 | 11 | 0.117 |                      |         |
|          | 60 | 45 | 0.768 |                      |         |
| PG       | 38 | 10 | 4.642 | 41.205 <sup>f</sup>  | 0.000*  |
|          | 52 | 11 | 8.085 |                      |         |
|          | 60 | 45 | 4.714 |                      |         |
| PGS      | 38 | 10 | 0.471 | 93.681 <sup>f</sup>  | 0.000*  |
|          | 52 | 11 | 0.832 |                      |         |
|          | 60 | 45 | 0.293 |                      |         |

## RESULTS

We did not observe any drumming behaviour in *N. leucodon* ( $n = 22$ ). In contrast, some individuals of *N. xanthodon* in each sampled locality displayed drumming behaviour (Table I). The numbers of individuals in Table I which displayed the drumming behaviour were 14, 11 and 45 for the cytotypes  $2n=38$ , 52 and 60, respectively.

The ANOVA test among the three cytotypes showed significant differences in all variables ( $p = 0.000$ ). When we performed a post-hoc test, the cytotype  $2n=60$  was statistically different from other cytotypes in terms of PU (signal velocity), with faster signal. While the PGS variable was different among all three cytotypes, the PG variable was the same for the  $2n=38$  and 60; but different for the  $2n=52$  cytotype (Table II). According to the PCA results the individuals with identical diploid numbers were grouped closely with each other in a two-dimensional space (Fig. 3). Comparisons of FN variations of the cytotypes  $2n=52$  ( $FN=72$  and 74) and 60 ( $FN=74$ , 78 and 80) did not show significant differences within  $2n=52$  and  $2n=60$  (Table III).



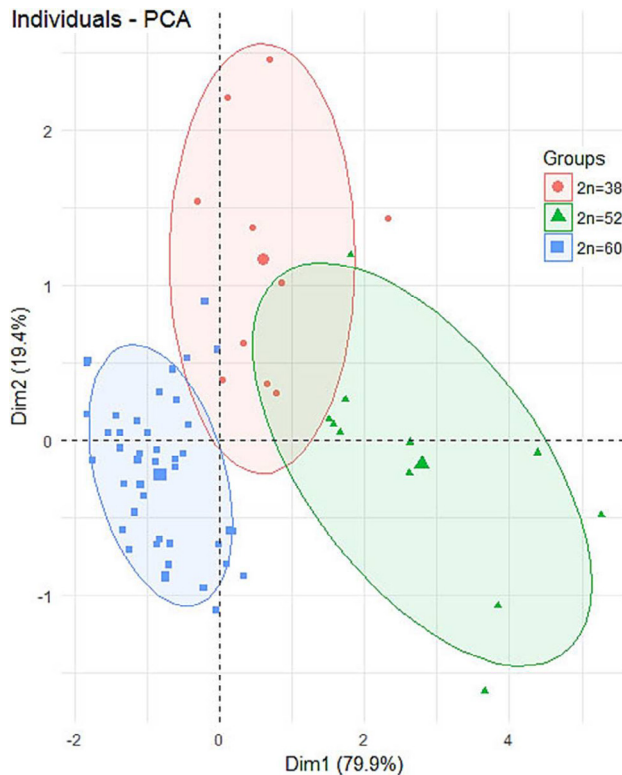


Fig. 3. Representation of individuals on a two-dimensional plane after PCA.

**Table III.- FN comparison (ANOVA shows the results of three characters among FN variations. Comparisons in  $2n=52$  was calculated by t-test, but F statistic was used for comparisons of three NF variation in  $2n=60$ . t: t value, f: F value).**

| Variable | 2n | FN | n  | Mean   | Test value          | p-value |
|----------|----|----|----|--------|---------------------|---------|
| PU       | 60 | 74 | 5  | 0.758  | 0.201 <sup>f</sup>  | 0.819   |
|          |    | 78 | 20 | 0.766  |                     |         |
|          |    | 80 | 15 | 0.783  |                     |         |
|          | 52 | 72 | 6  | 0.1121 | -1.729 <sup>t</sup> | 0.146   |
|          |    | 74 | 5  | 0.1234 |                     |         |
|          |    | 76 | 4  | 0.1345 |                     |         |
| PG       | 60 | 74 | 5  | 4.242  | 2.012 <sup>f</sup>  | 0.148   |
|          |    | 78 | 20 | 5.064  |                     |         |
|          |    | 80 | 15 | 4.632  |                     |         |
|          | 52 | 72 | 6  | 8.333  | 0.496 <sup>t</sup>  | 0.626   |
|          |    | 74 | 5  | 7.787  |                     |         |
|          |    | 76 | 4  | 7.123  |                     |         |
| PGS      | 60 | 74 | 5  | 0.245  | 2.042 <sup>f</sup>  | 0.144   |
|          |    | 78 | 20 | 0.318  |                     |         |
|          |    | 80 | 15 | 0.299  |                     |         |
|          | 52 | 72 | 6  | 0.805  | -0.392 <sup>t</sup> | 0.710   |
|          |    | 74 | 5  | 0.865  |                     |         |
|          |    | 76 | 4  | 0.912  |                     |         |

Among all three cytotypes, 13 out of the total of 60 individuals displaying drumming behaviours were evaluated for temporal variation. A total of 13 individuals (one with  $2n=38/NF=74$  and twelve with  $2n=60/FN=78$  were used for multiple measurements ( $>0.05$ ). Using repeated measurements tests for the same individuals, we found the difference in the peak number (PG) in only two individuals and no difference in the signal length (PGS). There was significant variation in the signal speed (PU) in all but two individuals.

We found no statistically significant difference in seismic signalling between sexes in all three variables (t-test, Table IV). For the ecological comparisons, t-test results showed no significant difference between the seismic signal patterns in the individuals from different soil and climate types (t-test, Table V).

**Table IV.- Sex comparison (t-test comparisons of sexes. t: t value).**

| Variable | 2n | F  | M  | Mean (female/male) | Test value          | p-value |
|----------|----|----|----|--------------------|---------------------|---------|
| PU       | 38 | 6  | 4  | 0.98/0.119         | -3.70 <sup>t</sup>  | 0.13    |
|          | 52 | 6  | 5  | 0.111/0.119        | -1.105 <sup>t</sup> | 0.298   |
|          | 60 | 25 | 20 | 0.79/0.77          | 0.645 <sup>t</sup>  | 0.523   |
| PG       | 38 | 6  | 4  | 0.510/0.420        | 1.254 <sup>t</sup>  | 0.245   |
|          | 52 | 6  | 5  | 0.792/0.739        | 0.546 <sup>t</sup>  | 0.598   |
|          | 60 | 25 | 20 | 0.294/0.284        | 0.423 <sup>t</sup>  | 0.675   |
| PGS      | 38 | 6  | 4  | 5.013/4.112        | 1.49 <sup>t</sup>   | 0.196   |
|          | 52 | 6  | 5  | 8.113/7.672        | 0.568 <sup>t</sup>  | 0.584   |
|          | 60 | 25 | 20 | 4.745/4.639        | 0.324 <sup>t</sup>  | 0.748   |

## DISCUSSION

Social communication has a vital role mediating behaviour such as mate choice, territoriality, and kin selection. Because mating between individuals of different species can have negative fitness consequences, animals are expected to be under strong selection to produce and recognize species-specific mating signals (Ryan and Rand, 1993). Thus, the signals used for species recognition should show less variation across environmental and season factors. This should be especially true for blind mole rats (*Nannospalax* sp.), which live underground with no visual sensory ability and limited opportunity for dispersal. Indeed, our results show that *N. xanthodon* differ significantly in drumming components by cytotype and lend support to previous studies that show individuals can recognize species through seismic signals.

**Table V.- Ecological comparisons (t-test between ecological groups. t: t value).**

| Variable | 2n | FN | Locality | n  | Mean  | Soil/Climate | Test value          | Sig   |
|----------|----|----|----------|----|-------|--------------|---------------------|-------|
| PU       | 60 | 78 | Kapaklı  | 15 | 0.770 | Sandy soil   | -0.740 <sup>t</sup> | 0.467 |
|          |    | 78 | Oklubal  | 10 | 0.80  | Clay soil    |                     |       |
|          |    | 80 | Gölbaşı  | 6  | 0.740 | Arid         | -0.943 <sup>t</sup> | 0.366 |
|          |    | 80 | Tavşanlı | 9  | 0.860 | Semi humid   |                     |       |
| PG       | 60 | 78 | Kapaklı  | 15 | 4.715 | Sandy soil   | -0.780 <sup>t</sup> | 0.444 |
|          |    | 78 | Oklubal  | 10 | 5.104 | Clay soil    |                     |       |
|          |    | 80 | Gölbaşı  | 6  | 4.517 | Arid         | 0.41 <sup>t</sup>   | 0.968 |
|          |    | 80 | Tavşanlı | 9  | 4.732 | Semi humid   |                     |       |
| PGS      | 60 | 78 | Kapaklı  | 15 | 0.285 | Sandy soil   | -1.026 <sup>t</sup> | 0.316 |
|          |    | 78 | Oklubal  | 10 | 0.324 | Clay soil    |                     |       |
|          |    | 80 | Gölbaşı  | 6  | 0.263 | Arid         | -0.492 <sup>t</sup> | 0.652 |
|          |    | 80 | Tavşanlı | 9  | 0.319 | Semi humid   |                     |       |

The lack of drumming behaviour in samples of *N. leucodon* appears to be quite interesting since the seismic signalling is reported for many member of family Spalacidae, in particular for communication purposes (Hrouzková *et al.*, 2013; Li *et al.*, 2001; Nevo *et al.*, 1987; Rado *et al.*, 1987). Our total sample size for the *N. leucodon* was 20 individuals and this number is higher compared to many other locations of *N. xanthodon* distributed throughout the Asian part of Turkey. At the same time, drumming behaviour was not observed in only one locality of the cytotype 2n=38. It could be that the reason for the absence of drumming behaviour in *N. leucodon* and 2n=38 cytotype of *N. xanthodon* is similar. We suggest that a new independent experiment might be needed to get precise information on the drumming behaviour in *N. leucodon*, perhaps using large samples from different locations.

The comparison between cytotypes and FN may provide evolutionary insights. Seismic signals could be useful in kinship recognition. The different seismic signalling patterns per each cytotype implied that individuals may use seismic signalling to recognize conspecifics. The behaviours of individuals towards each other may be related to their genetic relatedness both within and between populations. Since the seismic signalling variations among different cytotypes of Palestinian blind mole rats were shown to be used for recognition of conspecifics, it was interpreted as a contribution to reproductive isolation between the cytotypes (Heth *et al.*, 1987; Rado *et al.*, 1987). Our results show that each cytotype of *N. xanthodon* showed as a clear cluster on the individual principle components plot (Fig. 3). Almost 80% of the variation could be explained by the differences between 2n=60 and the other two cytotypes. Similar to *N. ehrenbergi*, the seismic signal patterns in *N. xanthodon* varied by diploid chromosome numbers and may represent the means of species-specific communication.

Interestingly, the first PC axis revealed closer relationship between the cytotypes 2n=38 and 2n=52, and a slightly tighter clustering of individuals with cytotype 2n=60 (Fig. 1). This corresponds to a recent finding by Matur *et al.* (2019), who showed that 2n=52 and 2n=38 belong to the same Western Anatolian phylogenetic clade, different from the Central Anatolian clade that contains 2n=60.

In the present study, we tested whether there is a difference in drumming patterns among individuals with different numbers of chromosome arms (FN) within the same cytotypes 2n=52 (FN=72 and 74) and 60 (FN=74, 78 and 80). Difference in FN variants may theoretically serve as a reproductive barrier between populations or mark population divergence. However, we found no statistically significant difference. This means that similar drumming signals are used by all individuals that belong to the same cytotypes and such similar signalling may prevent behavioural isolation among FN variants.

We found significant unexplained differences among the repeated measurements in the signal speed (PU) in most individuals. This result could not be caused by seasonal variation, since all recordings per each individual were performed within a short period of two months. Variation in diet regime is also an unlikely cause since all animals were given the same food *ad libitum*. It is interesting that some animals did not display temporal variation. We conclude that this finding needs further investigation. Other variables (PG and PGS, with minor exceptions) remained constant for the same individuals over the entire experiment. We suggest that the drumming signalling patterns may be used as an additional taxonomic character to differentiate between cytotypes. However, other factors may affect the signalling pattern. For example, the foot-drumming signals made by the kangaroo rats are affected by their social environment, and the variation is higher in juveniles than in adults (Randall and Matocq, 1997;

Randall, 1991, 1995). We could not determine the age of individuals, as methods for reliable age verification in BMRs are non-existent or poorly developed (Puzachenko, 1996). Therefore we cannot exclude the possibility that some portion of variation between cytotypes in our study was linked to age. However, age alone cannot account for the strong differences in signalling pattern between the cytotypes.

Although mate choice has been proposed as one explanation for species-specific communication, we found that seismic signal structures were similar for both sexes (Table IV). It appears that there is no sexual dimorphism in *N. xanthodon* in terms of seismic signal structures (Table IV) and thus it is unlikely that this communication is under sexual selection. Blind mole rats are known to be solitary animals, and only one individual occupies a single nest, except during mating season or maternal care (Nevo, 1961). They defend their territories against intruders and they use seismic signals to define the borders of their territories (Zuri and Terkel, 1996). Heth *et al.* (1987) reported that the Palestinian blind mole rat *N. ehrenbergii* use seismic signals for territorial purposes. Our results suggest that seismic signals may also be used for territorial purposes by the Anatolian blind mole rat *N. xanthodon*.

The drumming signals of animals originated from different soil and climate types did not show any difference (Table V). We conclude that ecological factors play little or no role in shaping the seismic signalling of *N. xanthodon*.

## CONCLUSION

The seismic signalling structure of two mole rats species of *Nannospalax* in Turkey evaluated in the manuscript. The *N. leucodon* has not drumming behaviour based on our samplings and needs elaborately study across its distribution range in Turkey. Although the cytotypes of *N. xanthodon* in Turkey have ecological, sex and chromosomal variations (like FN), they have unique seismic signalling structure. We gave a description of some races of seismic signalling patterns of mole rats in Turkey first time and it must be studied in detail in future in all *Nannospalax* cytotypes.

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## Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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