= GENERAL BIOLOGY ===

Acoustic Structure of Alarm Calls in Indian Sambar (*Rusa unicolor*) and Indian Muntjac (*Muntiacus vaginalis*) in South Vietnam

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Abstract—The alarm call acoustic structure and nonlinear vocal phenomena of the Indian sambar (*Rusa uni-color*) and northern Indian muntjac (*Muntiacus vaginalis*) have been analyzed in detail as well as their vocal behavior in response to mobbing humans under natural conditions of southern Vietnam. The alarm calls of sambars, tonal barks separated by large intervals, were produced by animals standing on the place and gazing at a potentially dangerous object. Muntjacs flee off in danger and produced a series of dull barks interrupted with short intervals from a distance. The alarm call frequencies were characterized for sambars and muntjacs. The results of our study have been compared with the published data on alarm calls of other Cervidae species.

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The alarm calls in response to a potential danger are widespread among mammals of many mammalian taxa. Their study is important for understanding the ways of transmission of semantically similar signals with information on a predator type [1], the ways of coding the degree of threat and the urgency of responding to it [2, 3], and for interspecific communication based on alarm calls of other species [4].

The best studied alarm calls are those of the colonial rodents, such as terrestrial squirrels, primates, and some other species. Ruminants represent a promising group for studying the vocal vigilance behavior: alarm calls are widely spread among various species of deer, antelopes, gazelles, and goats. However, few reports on their vocal behavior are available. In the Cervidae family, the alarm call acoustic structure has been analyzed only in five species: the white-tailed deer *Odocoileus virginianus* [5], sika deer *Cervus nippon* [6, 7], and European red deer *C. elaphus* [7–9], as well as Chinese and Indian muntjacs (*Muntiacus reevesi* [10] and *M. muntjac* [11]).

Indian sambar (Rusa unicolor) and northern Indian muntjac (M. vaginalis) are the most common ruminant species of southern Vietnam [12]. Both species stay in thickets in the daylight, and they go out for feeding on fields and forest glades at night. Sambar is strongly dependent on water, and it attends water bodies daily, while muntjac is dependent on water to a lesser extent. For the most part of the year, sambars and muntiacs keep themselves in small family groups or singly. They have no clearly expressed seasonal reproduction, harems are not formed; in sambars, less than a half of adult females are involved in reproduction every year. Near the human settlements, these animals stay commonly in the depth of forest. When they meet a human, their behavior is anxious and often accompanied with alarm calls [11, 12].

During this study, we analyzed the acoustic structure of the sambar and muntjac alarm calls which were recorded in natural environment of Vietnam.

The alarm calls of free-living deer have been recorded in the Cat Tiên National Park (southern Vietnam, $11^{\circ}21'-11^{\circ}48'$ N, $107^{\circ}10'-107^{\circ}34'$ E) in 2012–2016 from April to August. Most records were made in the pre-dawn twilight and for two hours after dawn. In all cases, a researcher who was lurking around or moved along the road saw the animal that has noticed him.

The sounds (48 kHz, 16 bits) were recorded using a Marantz PMD-660 (D&M Professional, Japan) professional digital recorder with a Sennheiser K6-ME66 directional condenser microphone (Sennheiser Electronic, Germany). The distance between the researcher and the animal ranged from 20 to 100 m.

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| Acoustic parameters | Sambars $(n = 18)$ | Muntjacs $(n = 14)$ |
|---------------------|--------------------|---------------------|
| Duration, s | 0.15 ± 0.03 | 0.24 ± 0.04 |
| Interval, s | 23.14 ± 10.05 | 6.77 ± 3.60 |
| f0beg, kHz | 0.80 ± 0.14 | 0.58 ± 0.04 |
| f0max, kHz | 0.98 ± 0.26 | 0.66 ± 0.08 |
| f0end, kHz | 0.64 ± 0.09 | 0.57 ± 0.06 |
| df0, kHz | 0.34 ± 0.28 | 0.10 ± 0.06 |
| fpeak, kHz | 1.61 ± 0.57 | 0.89 ± 0.28 |
| q25, kHz | 1.31 ± 0.25 | 1.00 ± 0.17 |
| q50, kHz | 1.95 ± 0.38 | 1.74 ± 0.32 |
| q75, kHz | 3.03 ± 0.59 | 2.93 ± 0.94 |

Acoustic parameters of alarm calls of Indian sambars and Indian muntjacs of southern Vietnam

 $M \pm SD$, *n* is the number of individuals studied.

Each record was regarded as that received from a separate animal. For sambars, we have obtained 19 records of alarm calls from 18 adult animals (the alarm call of one individual was recorded twice in different days). The sex has been determined in five individuals (one male and four female animals). In total, 45 alarm calls of sambars have been recorded and analyzed, from one to seven calls for each animal (on average, 2.50 ± 1.95 ; thereafter, $M \pm SD$). For muntjacs, we have received 14 records of alarm calls from 14 adult animals; the sex has been determined in five individuals (two male and three female animals). In total, 155 alarm calls have been recorded and analyzed for muntjacs, from 1 to 47 alarm calls per animal (11.07 ± 12.98).

The sounds were analyzed using the Avisoft SASLab Pro spectrographic software (Avisoft Bioacoustics, Germany). To remove the low-frequency noise, the sounds with frequencies lower than 100 Hz were filtered out. Sampling frequency was downsampled to 22.05 kHz. Spectrograms were constructed using the following characteristics: Hamming window frequency, fast Fourier transform length (FFT-length, 1024 points), overlapping along the frequency axis (frame, 50%), and overlapping on time axis (overlap, 96.87%). In the spectrogram window, the following parameters were measured for each sound: duration and, when possible, the initial (f0beg), maximum (f0max), and final (f0end) fundamental frequencies, as well as the interval to the next sound of the series. The smallest values of the initial and final fundamental frequencies were taken to be the minimum fundamental frequencies. The depth of frequency modulation (df0) was calculated as the difference between the maximum and minimum frequencies. In the window of the sound-averaged energy spectrum (mean power spectrum), the peak frequency (fpeak) was measured, as well as three quartiles of the spectrum (q25, q50, q50)q75) covering 25, 50, and 75% of the sound spectral power, respectively. In addition, the nonlinear vocal phenomena were spotted in sounds: deterministic chaos, subharmonics, and jumps of the fundamental frequency [13]. Since different numbers of alarm calls were recorded from different individuals, the mean values of acoustic parameters were calculated for each animal, and the results obtained were used to calculate the mean values for the whole species.

Sambar, as well as muntjac, produced alarm calls in danger, and this was characteristic of male and female animals of both species. Sambar and muntjac produced different alarm calls, and their behavior was different in response to arrival of a human.

In danger, sambars stood with its tail uplifted, and the animal was peering at the disturbing object. Standing at place, they were slowly raising one leg after another to kick strongly the substrate. This demonstrative behavior could continue for tens of minutes, and more than ten kicks could be made. The alarm calls produced through a wide open mouth of the sambar followed with large intervals of 23 s on average; this was accompanied by jerking of the animal tail (table). In the case of unexpected spotting of a human, the sambar was fleeing off with a single cry.

Muntjacs in danger first flew 30-70 m away or into thickets; after that, they stopped and shouted for a long time producing the alarm call series from several to several tens in a series. Intervals between the alarm calls of muntjacs were shorter than in sambars: 6-7 s on average (table). Three out of 14 muntjacs shouted while they were fleeing away of the source of danger; their alarm calls were produced as fast series with short intervals between them (0.85 ± 0.17 s).

The alarm calls of sambars were intense high-frequency tonal short barks (figure). The maximum fundamental frequency ranged in different individuals from 0.73 to 1.94 kHz (0.98 kHz on average (table)). Sambar barks had an arc-shaped structure; the depth of frequency modulation was 0.34 kHz. Nonlinear vocal phenomena were rare: deterministic chaos was recorded only in one alarm call produced by a single animal (2% of all barks); subharmonics in five barks (11%) from four animals. Jumps of the fundamental frequency were detected in alarm calls of two animals (a two- to threefold increase (Fig. 1)).

The alarm calls of muntjacs were intense noisy barks with a somewhat longer duration than in sambars (table, Fig. 1). Despite the smaller body size of muntjacs as compared to sambars, the values of maximum fundamental frequency were lower in muntjacs; they averaged about 0.66 kHz and ranged from 0.58 to 0.85 kHz in different individuals (table). In muntjac barks, the frequency modulation was much weaker expressed than in sambar barks. The peak frequency and quartile values were lower in muntjac barks than in those of sambars, suggesting a shift of acoustic energy into the low-frequency spectral bands in muntjacs. Deterministic chaos was normally occurring in most

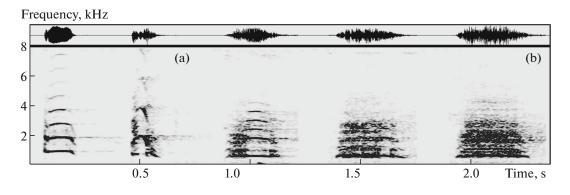


Fig. 1. Spectrograms (below) and oscillograms (above) of (a) two alarm calls of an Indian sambar and (b) three alarm calls of an Indian muntjac. A jump of the fundamental frequency can be seen in the second alarm call of the sambar. In three alarm calls of the muntjac, the deterministic chaos covers 40, 80, and 100% of the bark duration.

barks of all muntjacs (148 barks, 95% of all barks), and in 54 barks of five animals it completely masked the fundamental frequency course. Subharmonics were recorded in 16 barks of three individuals (10% of barks) and in 15 barks the subharmonics were recorded along with deterministic chaos. In addition, a short (for 0.04 ± 0.01 s), high-frequency (1.13 \pm 0.07 kHz) tonal start was in 17 alarm calls of two muntjacs.

In other deer species, acoustic analysis of alarm calls showed that they were either high-amplitude barks or clearly tonal (in sika deer and maral, a Siberian subspecies of red deer [6, 7, 9]), or dull noisy barks (in red deer of the Scottish subspecies, Indian and Chinese muntjacs [7, 10, 11]). Sika deer [6] and Indian muntjacs [11] produced alarm calls through a wide open mouth like sambars, while Canadian wapiti (a North American subspecies of the red deer) opened its mouth only slightly and somewhat extended the lips [8]. The alarm calls produced by white-tailed deer were unusual: they resembled an intense exhalation through an open mouth [5].

All of the cervine species studied usually produced alarm call series in which the number of barks could reach 100 and more [6, 9, 11]. In sika deer, Canadian wapiti, and Chinese muntjacs, as well as in sambars and Indian muntjacs, both male and female animals produced the alarm calls [6, 8, 10], although in females they were more common [7]. Typical sambar behavior (stamping the leg on substrate) has not been earlier described. It was also characteristic of Indian muntjacs in Nepal [11], but not of Vietnamese muntjacs in our study.

The alarm call duration that we have determined in the sambar and muntjac was similar to that in other deer species. It averages about 0.14 s in the white-tailed deer [5], was 0.12-0.17 s in the sika deer [6, 7], 0.20-0.25 s in the red deer [7–9], 0.26 s in the Indian muntjac from Nepal [11], and ranges from 0.27 to 0.51 s in different individuals of the Chinese muntjac [10]. The fundamental frequency of sambar alarm calls was significantly lower than that of the sika deer barks (f0max = 2.60-2.69 kHz, f0min = 1.66-1.82 kHz [6, 7]); this parameter was similar to the bark fundamental frequency of the Siberian subspecies of red deer (maral) (f0max = 0.93 kHz, f0min = 0.34 kHz [9]) and was much higher than in the Scottish subspecies of red deer (f0max = 0.15 kHz [7]). As determined in our study, the fundamental frequency of muntjac alarm calls was very close to that of Indian muntjacs from Nepal (f0max = 0.59 kHz, d.f. = 0.12 kHz [11]).

Although the sambar and muntjac inhabit the same habitats, the acoustic structures of their alarm calls are much different; the smaller deer species (muntjac) produces the alarm calls with a lower fundamental frequency than that of alarm calls of the sambar, the larger species. It was uncommon for mammals that there was no reverse correlation between body size and the alarm call fundamental frequency [14], but the same was also found in other deer species [6–11].

Both sambars and muntiacs produce bark-like alarm calls, but in published data only the muntjac is referred to as a barking deer [10, 11]. This may be a result of a greater noisiness of alarm calls in muntjac than in sambar, which makes the muntjac alarm calls similar to the barking of domestic dogs [15]. The alarm calls of the muntjac are produced with short intervals, they are organized in longer series and can be heard more often than those of the sambar. Nevertheless, sambar alarm calls propagate better in the environment, probably, because of a larger size of these animals rising over the grass level in which muntjacs are hidden almost completely; in addition, sambar alarm calls are more intense and are tonal in structure, while noisy barks of muntjacs are strongly absorbed by dense vegetation [10].

Thus, this study is the first to describe the alarm call acoustic structures of sambars and northern Indian muntjacs inhabiting southern Vietnam. Although both species live in similar biotopes, their alarm calls are species-specific and differ strongly. In sambars and muntjacs, the body size is negatively correlated with the fundamental frequency values of their alarm calls, which is uncommon for mammals in general but may be characteristic of the entire Cervidae family. Further comparative species analysis of the alarm call structure in ruminants is required to determine general and species-specific coding of information on animal arousal in response to potential danger.

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