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Research Article

The Female Reproductive Cycle of the Bedriaga Plate-Tailed Gecko, *Teratoscincus bedriagai* (Sauria: Gekkonidae) in Iran

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The Bedriaga Plate-tailed Gecko, *Teratoscincus bedriagai* Nikolsky, 1900, is distributed in the northern and eastern desert basins of the Central Plateau of Iran, Sistan, and the desert regions of southern Afghanistan. Iranian specimens are believed to be rare in collections. In this study, the reproductive cycle of this species has been investigated through focusing on oogenesis from 5 April to 5 August, 2013. Generally, 15 adult females were collected by hand at midnight from southern parts of Damghan County, situated in Semnan Province of Iran. Ovaries were removed and processed for the purpose of histological and morphometric studies. The results revealed that oocyte growth starts in early April and terminates in late July. Moreover, mating commences in spring, especially at the beginning of May, with oviposition occurring from late May to late July. Approximately, 1 to 2 eggs are laid by females per clutch with the possibility of producing a secondary clutch later in the season. The maximum reproductive activity takes place in May and continues with a decreasing trend in June and more reduction in July and finally ends in August. No significant difference was observed between right and left side of reproductive system. Therefore, oogenesis occurs from April to July, while *T. bedriagai* follows an oogenic cycle typical for temperate species.

1. Introduction

Lizards show three general types of reproductive cycles: constant, associated, and dissociated [1]. A constant reproductive cycle is exhibited by tropical lizards in which gonads are active almost year-round [2]. Associated and dissociated reproductive cycles are characterized by the presence of a discontinuous mating season. In associated type, gonadal activity increases immediately prior to the mating period in both males and females simultaneously and females have no need to store sperm due to its availability during the reproductive season. This cycle is common in species that live in predictable environments such as in the temperate zones [3], the subtropics [4], and the seasonal tropics [5]. In the dissociated type, gonadal activity is low during the mating period and peaks during the nonmating period and male gonadal activity is shorter than that of females and sperm is stored by the female genital system for some months with fertilization occurring later [6]. A dissociated cycle is typically observed in species that live in temperate zones and have a brief mating season [7].

The Gekkota are an infraorder of reptiles, comprising all geckos and the limbless Pygopodidae. Gekkota are a suborder of Squamata and consist of seven families: [8–12] Gekkonidae, Carphodactylidae, Diplodactylidae, Eublepharidae, Phyllodactylidae, Pygopodidae, and Sphaerodactylidae [8, 10, 11].

The Plate-tailed Gecko or Skink Gecko, *Teratoscincus bedriagai* Nikolsky, 1900, belongs to the family Gekkonidae. In fact, *Teratoscincus* has long been a genus that was distinctive in morphological studies [13], and recent molecular work has placed it with the expanded family Sphaerodactylidae rather than the Gekkonidae [8, 10]. *T. bedriagai* is a nocturnal, insectivorous, and oviparous lizard distributed in the northern and eastern desert basins of the Central Plateau of Iran, Sistan, and the desert regions of southern Afghanistan as far east as Kandahar. Iranian specimens are rare in collections. This species in Iran is distributed in Sistan and Balutchestan,

Figure 1: Map showing the sampling site (Damghan County) in northern Iran.

Khorasan, Semnan, Tehran and Yazd Provinces [14]. Since the time when Zarudny collected the types in 1898, the species was not found again until the 1962-63 Street Expedition, when a single specimen was collected near Zabol and Ranck along with the mammal survey of the National Museum of Natural History (Washington), when two specimens were collected by Hermann from the Sistan area in 1962 [15]. Furthermore, 10 specimens were collected by Tuck and Anderson from the Sistan area in 1975 [14]. A description of this species was presented by Anderson [14] which was based on five specimens. Further morphological data in accordance with 50 specimens collected in the Semnan Province of Iran was given by Hojati [16]. Since no data is available on the reproduction of this species, this research has been conducted to characterize the female reproductive cycle of this species in Iran.

2. Materials and Methods

2.1. Study Area. All specimens were collected from Hassan Abad, Saleh Abad, Alian and Yazdan Abad villages located in south of Damghan County, Semnan Province of Iran (54°19′E, 35°55′N). Damghan is located at 1170 m above sea level and north of the Central Kavir Desert (Figure 1). The annual average temperature is 17.2°C. The study area consists of alkaline saline soils comprising clay and sand. The dominant plant species are *Tamarix* sp., *Salsola* sp., *Alhaji* sp., *Peganum* sp., *Atriplex* sp., and *Astragalus* sp.

2.2. Sampling. Since there is an ethic to collect rare reptiles for science in the country, we used the permission of scientific collection from the Department of Environment of Damghan County to collect three adult specimens in each month.

Sampling was conducted periodically every month during the activity period of this species from 5 April to 5 August, 2013. All specimen collections were by hand incorporating a torch at night time (Figure 2). Generally, 15 adult and mature females were captured (three specimens per sampling period). Our observations revealed that females reached sexual maturity at a body length (SVL) of about 55 mm, while we tried to capture specimens which had attained larger SVL to ensure that they were sexually mature adults. Some specimens were kept in terrarium for the purpose of studying the frequency of clutch deposition.

2.3. Methods. The specimens were transferred alive to the Zoology Laboratory of Islamic Azad University, Damghan Branch, and measurements of W (weight), SVL (south-vent length), TL (tail length), and HL (head length) were conducted accordingly. Then, they were anaesthetized by chloroform and anatomized. Next, the ROD (right ovary diameter), LOD (left ovary diameter), ROW (right ovary weight), LOW (left ovary weight), RFN (right follicles numbers), LFN (left follicles numbers), MaxRFD (right follicles maximum diameter), MaxLFD (left follicles maximum diameter), MinRFD (right follicles minimum diameter), MinLFD (left follicles minimum diameter), MRFD (right follicles mean diameter), MLFD (left follicles mean diameter), OEL (oviductal egg length), OEWi (oviductal egg width), OEW (oviductal egg weight), and FLD (follicular layer diameter) were measured. Length, width, and diameter were measured through a dial caliper having an accuracy of 0.02 mm. Weight was measured by a scale with an accuracy of 0.001 g. By the time Gonads were removed, they were inspected for metric and meristic characters. The number, weight, diameter (length and width), and volume of immature, growing, and mature follicles and oviductal eggs were investigated in right and left ovaries, separately. After fixing the ovaries in 10% formalin, tissues were dehydrated, cleared in Xylene, infiltrated, and embedded with paraffin. After that, sections were made at 5 to 7 microns, deparaffinized, rehydrated, stained (Hematoxylin and Eosin), and mounted. Last, the sections were studied using a light microscopy at 100x and 400x magnification and also photographs were prepared by a digital camera. Finally, data was analyzed hiring the SPSS 18 software, one-way ANOVA, and Tukey's test to compare biometric data among monthly samples ($P < 0.05$).

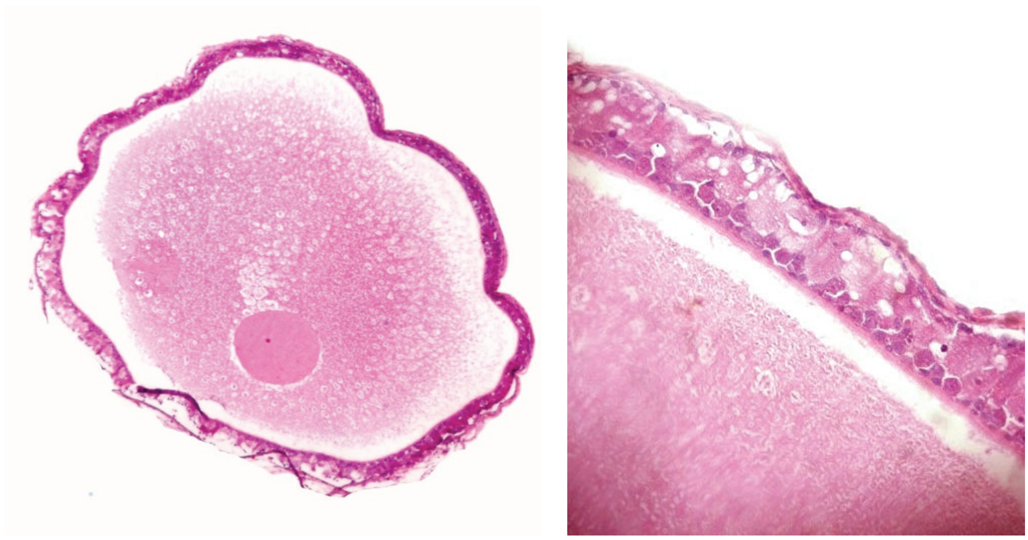
3. Results

In this study, the maximum SVL, TL, HL, and HW of samples were 68.62, 37.14, 17.14, and 15.89 mm, respectively. Also, the body length of the smallest mature female was 55 mm.

T. bedriagai speciesin the study area hibernates from early October to late March and early April. Generally, the specimens emerge in early April and begin oogenesis, whereas mating is observed in early May. The ovaries are paired and vesicular and consist of 3 to 8 follicles. Three types of follicles were observed: immature, growing, and mature. The growing follicles were observed in April. There was an immediate increase in ovary size in May and mature follicles were examined from early May to the mid of June. The mean diameter of ovary in April, May, and June was 2.32, 5.09, and 4.11 mm, respectively.

The follicular layer is multilayered and polymorphic (Figure 3). The mean diameter of follicular layer is 54.05 μm. Moreover, the diameter of the nucleus fluctuates from 27.83 to 45.50 μm in immature and growing follicles. The nucleoli are distinguishable from April to June and their numbers vary from 3 to 15 in immature and growing follicles, respectively. There seems to be an increase in the activity of nucleoli after

Figure 2: *Teratoscincus bedriagai* in Hassan Abad, Iran.



(a) (b)

Figure 3: The growing follicle (a) and follicular layer (b) of *Teratoscincus bedriagai* on 5 June.

Figure 4: *Teratoscincus bedriagai* on 5 May.

June and, then, they disappear in the nucleus. Finally, mature oocytes enter the oviducts, where fertilization occurs.

Also, oviductal eggs were observed between 5 May and 5 July (Figure 4). The weight, length, and width of the largest oviductal egg were 0.96 g, 14.50 mm, and 9.20 mm, respectively. Based on our observations, oviposition might take place twice a year. Oviposition occurs from late May to late July and the number of laid eggs varied from 1 to 2 per clutch. In the terrarium, the first two eggs were laid on 23 May (Figure 4).

White color and an oval shape are the two main features of eggs that were deposited from late May to late July. The egg shells are initially soft, but then they harden after 8–12 hours after deposition.

The weight, SVL, HL, and TL of the smallest hatchling obtained on 5 August were 2.29 g, 36.73 mm, 10.84 mm, and 19.96 mm, respectively. The juveniles were commonly investigated from July to September.

The descriptive statistics of macroscopic and microscopic characters are given in Tables 1 and 2, respectively. Analysis of variance of characters indicates that some characters including mean diameter of left and right ovaries and maximum, minimum, and mean diameter of follicles and follicular layer were significantly different among groups ($P < 0.05$). The maximum activity of oogenesis takes place in May (Figures 5 and 6).

Table 1: Descriptive statistics of macroscopic characters in *T. bedriagai* (for abbreviations, see Materials and Methods section).

Characters	N	Range	Minimum	Maximum	Mean		Std. deviation	Variance
					Statistic	Std. error		
W (g)	15	5.02	5.99	11.01	7.89	0.445	1.72	2.97
SVL (mm)	15	13.62	55.00	68.62	63.24	1.053	4.08	16.64
TL (mm)	15	14.39	22.75	37.14	31.21	0.94	3.63	13.18
HL (mm)	15	2.78	14.36	17.14	15.53	0.22	0.85	0.72
HW (mm)	15	4.80	11.09	15.89	13.46	0.372	1.44	2.08
ROD (mm)	15	3.20	2.10	5.30	3.85	0.27	1.06	1.13
LOD (mm)	15	3.49	2.10	5.59	3.97	0.26	0.99	0.99
ROW (g)	15	1.19	0.006	1.20	0.32	0.098	0.38	0.14
LOW (g)	15	1.44	0.006	1.45	0.36	0.11	0.42	0.18
OEN	3	0.00	2.00	2.00	2.00	0.00	0.00	0.00
OEL (mm)	3	8.08	6.42	14.50	9.67	2.46	4.27	18.19
OEWi (mm)	3	3.02	6.18	9.20	7.19	1.003	1.74	3.02
OEW (g)	3	0.18	0.78	0.96	0.84	0.04	0.07	0.005

Table 2: Descriptive statistics of microscopic characters in *T. bedriagai*.

Characters	N	Range	Minimum	Maximum	Mean		Std. deviation	Variance
					Statistic	Std. error		
RFN	15	4.00	4.00	8.00	5.67	0.37	1.45	2.09
LFN	15	5.00	3.00	8.00	5.87	0.39	1.51	2.27
Max RFD (mm)	15	0.80	1.70	2.50	2.09	0.07	0.28	0.08
Max LFD (mm)	15	0.93	1.75	2.68	2.23	0.08	0.30	0.09
Min RFD (mm)	15	0.99	0.85	1.84	1.32	0.08	0.32	0.10
Min RFD (mm)	15	1.30	0.74	2.04	1.35	0.10	0.38	0.14
MRFD (mm)	15	1.80	1.54	3.34	2.32	0.14	0.56	0.31
MLFD (mm)	15	2.76	1.20	3.96	2.36	0.20	0.79	0.62
FLD (μm)	15	29.90	40.60	70.50	54.05	2.68	10.38	107.79

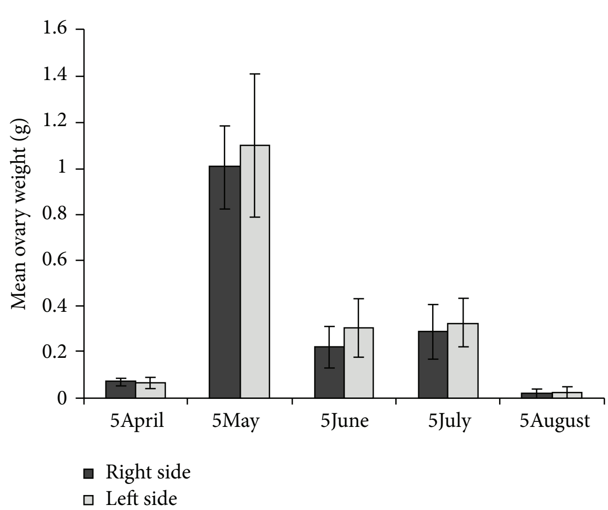


Figure 5: The mean ovary weight of *Teratoscincus bedriagai*.

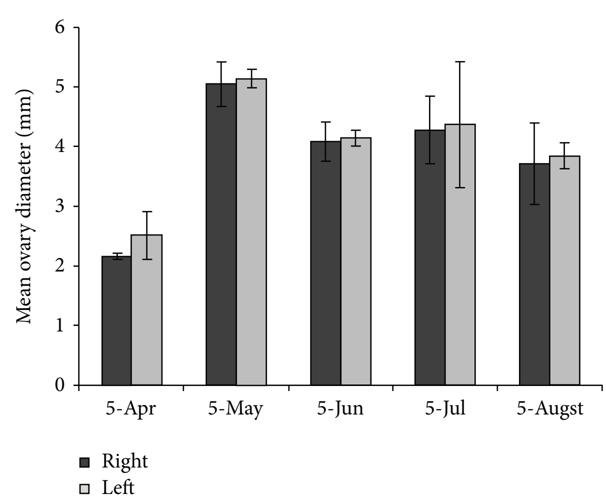


Figure 6: The mean ovary diameter of *Teratoscincus bedriagai*.

In addition, no significant difference is observed in the ovarian characters between the left and right side of the body (paired t-test, $P > 0.05$ in all cases).

4. Discussion

The significance of this study is owed to the fact that the results of this study regarding the oogenic cycle of *T. bedriagai* are being reported for the first time from Iran and west southern Asia. Since oogenesis occurred from April to July, the pattern followed an associated reproductive cycle typical for species from temperate areas.

Regarding the oogenic cycle of *T. bedriagai*, the three following phases have been reported according to the results of this investigation: (1) inactive phase: with small follicles from August to March, (2) vitellogenic phase: with growing and mature oocytes from early April to mid-May, and (3) oviductal egg phase from late May to mid-July.

There exists an increase in the weight and diameter of the ovaries and oocytes in May and the females tend to be heavier in mid- and late spring as a result of the presence of eggs. A similar clutch size was observed for this species compared to other gekkonid lizards in Iran like *Tenuidactylus caspius* [17] and *Cyrtopodion scabrum* [14]. According to the study conducted on the gecko *Hemidactylus mabouia*, the small cells of the granulosa layer are categorized into three separate cell types: small, intermediate, and the large pyriform cells [18]. A multilayer and polymorphic follicular layer was observed for *T. bedriagai* similar to other geckos such as *Tenuidactylus caspius* [17]. The general squamata pattern belonging to oviparous reptiles is similarly reported for the gross morphology, oogenesis, and folliculogenesis of the ovaries of *T. bedriagai* [18]. Almost 1 to 2 eggs per clutch are laid by this species, where the second egg is usually laid 2 to 7 days after the first one. Besides, the comparative analysis of egg shape for geckos has another advantage: invariant clutches of one or two eggs are laid by the female geckos of all species [19]. Hence, no more than one developing egg in a single ovary is owned by female geckos at a time.

Hardening of the egg shells takes place 8–12 hours after deposition and, therefore, their safety against desiccating environmental conditions and predators will be ensured compared to a soft shell. The following four gekkotan families, namely, Carphodactylidae, Diplodactylidae, Eublepharidae, and Pygopodidae, lay soft-shelled eggs, whereas their close relatives (Gekkonidae) lay hard-shelled eggs [20]. Production of this highly mineralized egg shell is not economical since calcium is a limiting element for most terrestrial organisms, but more elongated eggs are laid by small species within gekkonids compared to larger ones [21]. In this study, more than one egg clutch per year has been reported. Probably, hatching in *T. bedriagai* similar to other sympatric geckos, *Tenuidactylus caspius*, occurred after about 45 to 50 days after oviposition in July or early August.

Also, the results highlight an associated type reproductive cycle for *T. bedriagai* which occurs during the well-defined period in which oocytes were not found in the ovaries throughout the year. A continuous reproductive cycle overall a year with laying two eggs per clutch is observed for the geckos of tropic regions such as *Dixonius siamensis* [22], *Gekko gecko* [23], *Gekko smithii* [24, 25], *Cyrtodactylus malayanus,* and *Cyrtodactylus pubisulcus* [26]. Moreover, egg production in common barking gecko, *Ptenopus garrulus*, is limited to the right ovary in South Africa [27], while both ovaries participate in egg production in *T. bedriagai* species. The ovarian cycle and egg production are a function of temperature and photoperiod [28, 29]. Still, egg weight turns out to be a function of the female body weight and not her body length [30].

The pelvic limitation hypothesis predicts that lizards producing relatively larger eggs are forced to increase egg length rather than egg width. In geckos, this restriction would mean that lineages with soft-versus-hard eggshells should differ in egg length, but not in egg width relative to body size. Preliminary analysis shows that egg widths relate to SVL more or less equally in all gecko families with similar body shape.

Furthermore, the truly large distinction in scaling of egg size to SVL between gekkonids and eublepharids + diplodactylids + carphodactylids is limited to egg length. These results, thus, strongly support the pelvic limitation hypothesis on gekkonid egg shape. The relation between egg width egg length and SVL is isometric; that is, eggs grow proportionally to SVL, in eublepharids as well as in diplodactylids and carphodactylids sharing the same allometry. Therefore, we can conclude that egg length and consequently whole egg size in gekkonids reflect negative allometry; that is, the miniature gekkonids enlarge eggs by means of relative egg length increments. The oviductal eggs inside female eublepharids, carphodactylids, and diplodactylids are positioned nearly side by side. In contrast, gekkonid lizards with two eggs per clutch place their eggs one in front of the other [20]. The differences in relative egg size and egg position together with the observation that female eublepharids are anorexic several days before parturition whereas those of at least one gekkonid species (*Paroedura picta*) were observed feeding just several hours before laying suggest that the body cavities of eublepharids, diplodactylids, and carphodactylids before laying are more full. The abdominal volume, which increases in proportion to body size and then constrains relative clutch mass in diplodactylids, carphodactylids, and eublepharids, and the resultant egg size allometry in these groups are then isometric, a trait unique among ectotherms. On the contrary, empty space in the body cavity allows small female gekkonid lizards to enlarge eggs, but the pelvic limitation ensures that this only occurs by means of egg elongation [21].

The relationships between reproductive cycles and climate suggest that reproduction in lizards is affected by environmental variables such as temperature [31], precipitation [32], and photoperiod [33]. Phylogenetic constraints may also play a major role in shaping the reproductive characteristics of lizards [34]. If so, it may be beneficial to study the reproductive cycles of species within a single diverse and wide-ranging lineage.

Conflict of Interests

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

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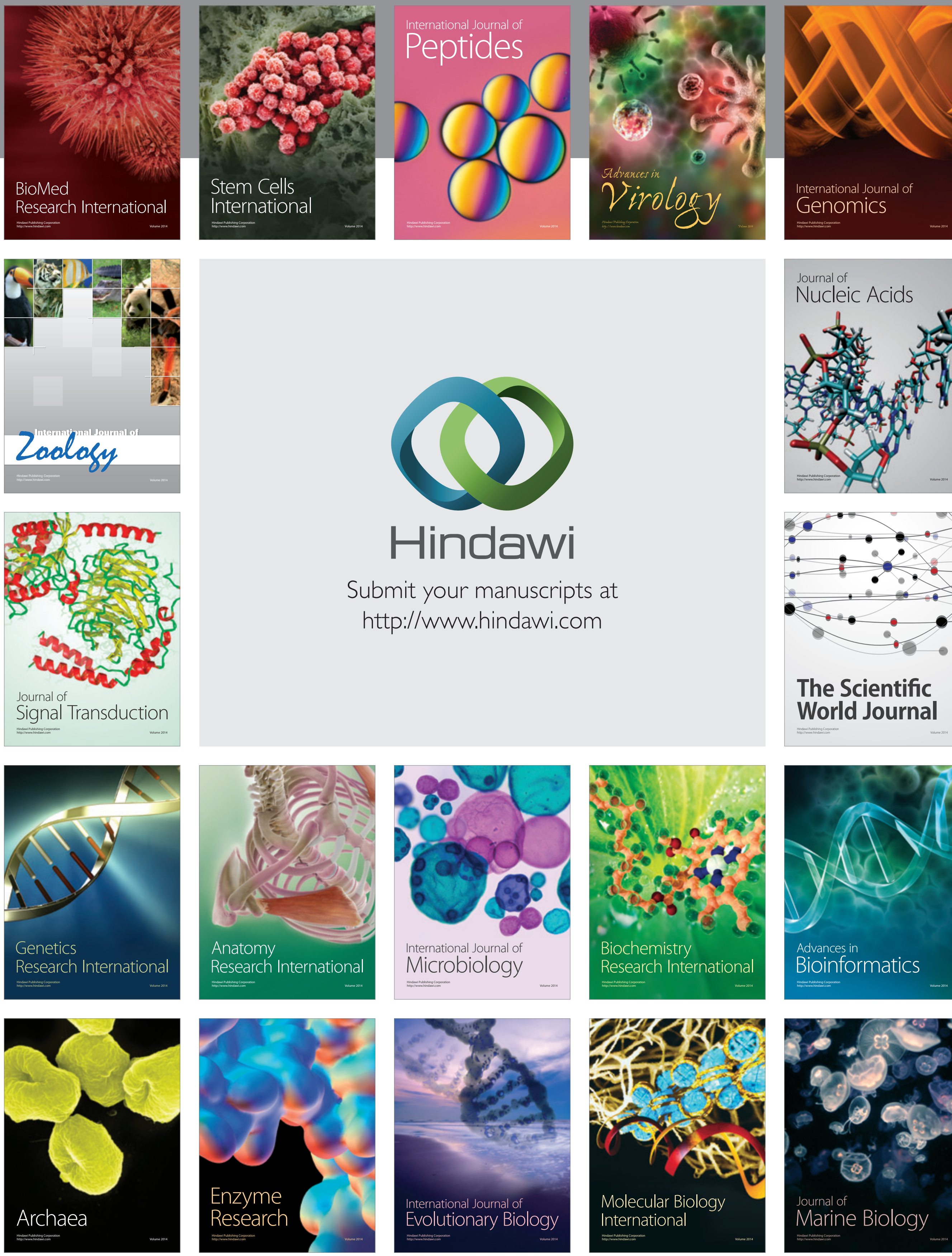
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