



**Full Length Article**

## Faunistic and DNA Barcoding of the Recorded Spiders (Araneae: Gnaphosidae, Hersiliidae and Salticidae) from Lower Dir (Pakistan)

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### Abstract

Spiders of family Gnaphosidae Pocock (1898), Hersiliidae Thorell (1870) and Salticidae Blackwall (1841) were collected from Dir Lower Pakistan and were preserved in ethanol. Preserved specimens were studied under stereomicroscope and photographed with the help of camera mounted on compound microscope. New faunistic records for three species *Micaria dives* Lucas (1846), *Hersilia savignyi* Lucas (1836) and *Cyrba ocellata* Kroneberg (1875) are provided from the country with brief description, digital photographs of external morphology as well as genitalia. While an increased distribution ranges of three species *Plexippus paykulli* Audouin (1826), *Menemerus nigli* Wesolowska and Freudenschuss (2012) and *Thyene imperialis* Rossi (1846) that is already reported from Pakistan. DNA base identification for all species is provided. COI sequences >200 bp recovered from the specimens were analyzed using neighbor-joining trees and Barcode Index Numbers (BINs) Nucleotide alignment for each species is provided with Process id, Sample id, match BIN and match process id. Arc GIS (10.5) was used to show the distribution map of the species in various localities. This study will impel and will help the taxonomist for future work on unknown biodiversity of Pakistan. It also provides molecular data of the recorded species for the first time from the country. © 2022 Friends Science Publishers

**Keywords:** Dir lower; Taxonomy; New record; Diversity; COI; Spiders

### Introduction

Salticidae represents more than 650 genera comprises of more than 6000 species from around the world (World Spider Catalog 2020). It is the most diverse family represents a greater number of genera and species. Many countries belong to Oriental region have very few species reported from (Seyfulina *et al.* 2020). From Pakistan the family has few reports (Ali *et al.* 2018). Number of species of the family are 74 from Pakistan till date (World Spider Catalog 2020). Previous work on the family is done by (Simon 1901; Reimoser 1934; Reimoser 1935; Caporiacco 1935b; Dyal 1935; Mushtaq *et al.* 1995a; Mushtaq *et al.* 1995b; Butt and Beg 2000; Azarkina 2004b, 2006; Butt and Siraj 2006; Wesolowska and Freudenschuss 2012; Ali *et al.* 2018; Sajid *et al.* 2020a; Sajid *et al.* 2020b).

Gnaphosidae Pocock 1898 comprises of more than 150 valid genera and more than 2500 species. Very few records of the family from Pakistan include, *Berlandina afghani* (Marusik *et al.* 2014b), *Berlandia drassodea*

(Caporiacco 1934a), *Drassodes involutus* (Pickard-Cambridge 1885), *Drassodes lutescens* (Koch 1839), *Drassodes rubicundulus* (Caporiacco 1934a), *Gnaphosa dege* (Ovtsharenko *et al.* 1992), *Gnaphosa pakistanica* (Ovtsharenko *et al.* 2008), *Leptopilos memorialis* (Spassky 1940), *Micaria dives concolor* (Caporiacco 1935b), *M. lenzi* (Bösenberg 1899), *M. pulcherrima* (Caporiacco 1935b), *Odontodrasus mundulus* (Pickard-Cambridge 1872), *Parasyrisca alai* (Ovtsharenko *et al.* 1994), *Prodidomus margala* (Platnick 1976f), *Synaphosus neali* (Ovtsharenko *et al.* 1994), *Talanites tibialis* (Caporiacco 1934), *Zelotes baltistanus* (Caporiacco 1934), *Zelotus faisalabadensis* (Butt and Beg 2004), *Zelotus illustris* (Butt and Beg 2004), *Zelotus pakistaniensis* (Butt and Beg 2004), *Zelotus pulchellus* (Butt and Beg 2004), *Zelotes sarawakensis* (Thorell 1890), *Zelotes sindi* (Caporiacco 1934). Family Hersiliidae (Thorell 1870) contain more than 15 genera and more than 180 valid species. No known species of the family is reported from Pakistan (World Spider Catalog 2020).

The objectives of the current study are (1) to provide three new species record to the fauna of spiders (Araneae) of Pakistan and (2) to provide images and illustrations for the new records (3) to provide data on distributional ranges of already reported species from the Northern most part of the country with Process id, Sample id, match BIN and match process id for Nucleotide sequence alignments of COI-5P.

## Materials and Methods

### Study area

The current study and collection were conducted from April 2018 to November 2019 in District Dir lower. Dir is a small former princely state situated in North of Khyber Pakhtunkhwa, 34° North latitude and 71° East latitude. Swat valley is situated on the East of Dir lower, Chitral valley at North, Bajaur and Afghanistan at west and Malakand district on its South (Sajid *et al.* 2020) Fig. 47.

### Collection and preservation

Collection was done from different localities including Hill and mountains, planes, marshes, river side's, leaf letters and under stones. Different methods like aerial hand collection, vegetation beating, cryptic searching and ground hand collection were used for spider's collection. Different sized vials were used for preservation, for larger spiders, large jars were used while for smaller spider's eppendorf tubes were used. Spiders were preserved in 95% ethanol. Vials were labeled with a number and specific information's of importance like altitude, latitude and longitude were recorded. All the measurements are given in millimeters.

### Identification

Identification was done by studying the spiders under LABOMED INC Los Angelis stereomicroscope (LB-344) at Department of Geology University of Swat, Khyber Pakhtunkhwa, Pakistan. Genitalia of both males and females specimens were photographed with Olympus DP71 attached to Olympus CX41 compound microscope at Academic Block College of Veterinary-Sciences Faculty of Animal-Husbandry and Veterinary-Sciences (Sajid *et al.* 2020). Specimens were identified with the help of available keys and literature i.e. (Zabka 1985; Davies and Zabka 1989; Caleb *et al.* 2017; Wesolowska 1996; World Spider Catalog 2020). The studied specimens are kept in Museum of Zoology Department Islamia College University Peshawar.

### Molecular study

Specimens were sent for DNA barcoding to the Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Canada. DNA extraction, PCR and

sequencing were performed with using Standard protocol (<http://ccdb.ca/resources/>) (Ashfaq *et al.* 2019). Nucleotide sequence alignments of COI-5P are generated by using Codon Code aligner version 8.02.

**Abbreviations:** BL. Body length, CL. Carapace length, CW. Carapace width, AL. abdomen length, AW. Abdomen width, RTA. Retrolateral tibial apophysis, TA, tegular apophysis, E. embolus. KP. Khyber Pakhtunkhwa.

## Results

In present collection one species *M. dives* (Lucas 1846) of the family Gnaphosidae, one species *Hersilia savignyi* (Lucas 1836) of family Hersiliidae and one species *Cyrba ocellata* (Kroneberg 1875) of the family Salticidae were reported for the first time from Pakistan.

### Family Gnaphosidae Pocock 1898

#### Genus *Micaria* Westring 1851

##### *M. dives* Lucas 1846

Figs. 1–11

*Drassus dives* Lucas, 1846: 220, pl. 13, f. 9 (Df).

*Micariolepis dives* Simon 1897a: 175.

*M. dives* Bösenberg 1902: 287, pl. 27, f. 421 (mf).

*M. dives* Caleb 2018a: 51, f. 1–8 (mf).

**Material studied:** Pakistan, KP, Talash Dir Lower, **Nagri Payeen** (34.74606E, 71.94049N, 1001m), 1♂1♀, 13.viii.2018, M. Sajid, Pakistan, KP, Talash Dir Lower, **Nagri Payeen** (34.74630E, 71.94024N, 1014m), 3♂4♀, 30.viii.2018, M. Sajid.

**Measurement:** BL.2.4–2.9, CL.1.1–1.6, CW. 1–1.2, AL. 1.3–1.8, AW. 1–1.2. legs formula is 4123. Leg length: I 2.43, II 2.36, III 2.02, IV 3.3.

**Distribution:** Central Asia, Caucasus, China, Europe, (Europe to Far East), India, Israel, Korea, Japan, Russia, Turkey (World Spider Catalog 2020). While present study extend it ranges to Northern part of Pakistan.

### Family Hersiliidae Thorell 1870

#### Genus *Hersilia* Audouin 1826

##### *H. savignyi* Lucas 1836

Figs. 12–20

*H. savignyi* Lucas 1836a: 10, pl. 13, f. 1 (Df).

*H. savignyi* Caleb *et al.* 2017: 396, f. 1–3 (mf).

**Sample id:** ICUP-MS-0080

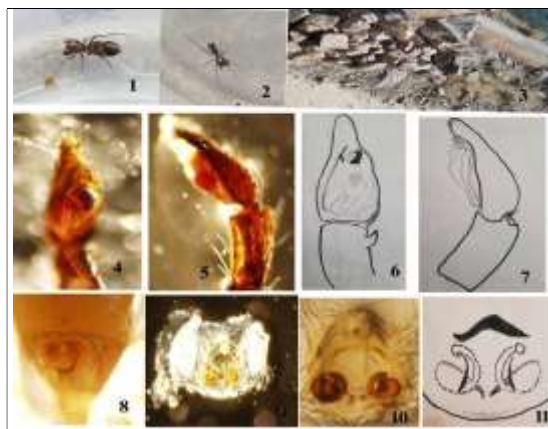
**Process id:** SPKPK080-20

**Match BIN:** BOLD:AAP4789

**Match Process:** MASPD659-10

**Materials studied:** Pakistan, KP, Talash Dir Lower, **Barcharay hill**, (34.71626E, 71.91669N, 1203m), 2♂3♀, 18.vi.2018, M. Sajid, Pakistan, KP, Dir Lower, **Goro hill**, (34.44714E, 71.94321N, 768m), 4♂2♀, 9.vii.2018, M. Sajid, Pakistan, KP, Talash Dir Lower, **Nagri Payeen** (34.74226E, 71.93949N, 983m), 1♂3♀, 25.vii.2018, M. Sajid, Pakistan, KP, Dir Lower, **Pato hill**, (34.76404E, 71.92481N, 1252m), 1♀, 9.vii.2018, M. Sajid.

**Measurement:** BL. 5.85–6.5, CL. 2.3–2.5, CW. 2.3–2.5, AL. 3.55–4, AW. 2.5–2.9. Legs formula. 1243. Legs length.



**Fig. 1-11:** *M. dives* Lucas (1846), 1, female; 2, Male; 3, Habitat; 4-6, Ventral view of male palp; 5-7, retrolateral view of palp; 8-9, ventral of female epigyne; 10-11, dorsal view epigyne



**Figs. 12-20:** *H. savignyi* Lucas (1836): 12-13, male and female habitus; 14, habitat; 15, left palp; 16, palp ventral view; 17, retrolateral view; 18-19, ventral view female epigyne; 20, dorsal view



**Figs. 21-30:** *C. ocellata* Kroneberg (1875): 21-22, male habitus; 23-24, female habitus; 25-26, habitat; 27, ventral view of male palp; 28, retrolateral view of male palp; 29, ventral view of female epigyne; 30, dorsal view

Legs: I: 34.1 mm; II: 30.5 mm; III: 9.5 mm; IV: 25.3 mm.

**Distribution:** Sri Lanka, India to Philippines (World Spider Catalog 2020). The species is previously some ambiguous report from the Pakistan but with no detail of photographs

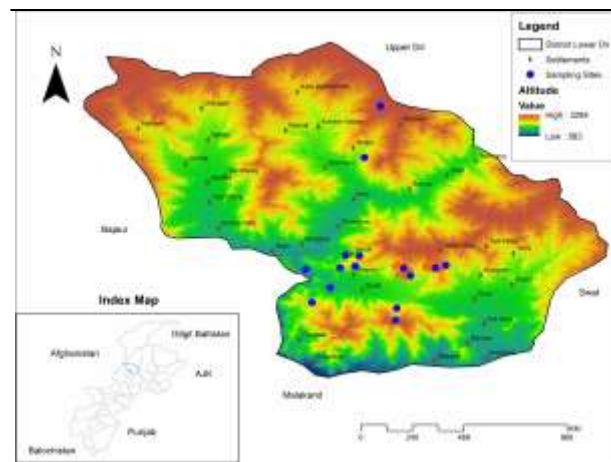
and images of genitalia. Present study provides detail of the species and confirms its existence from western part of Asia.

**Family Salticidae Blackwall 1841**

***Cyrrba ocellata* Kroneberg 1875** (Figs. 21-30)



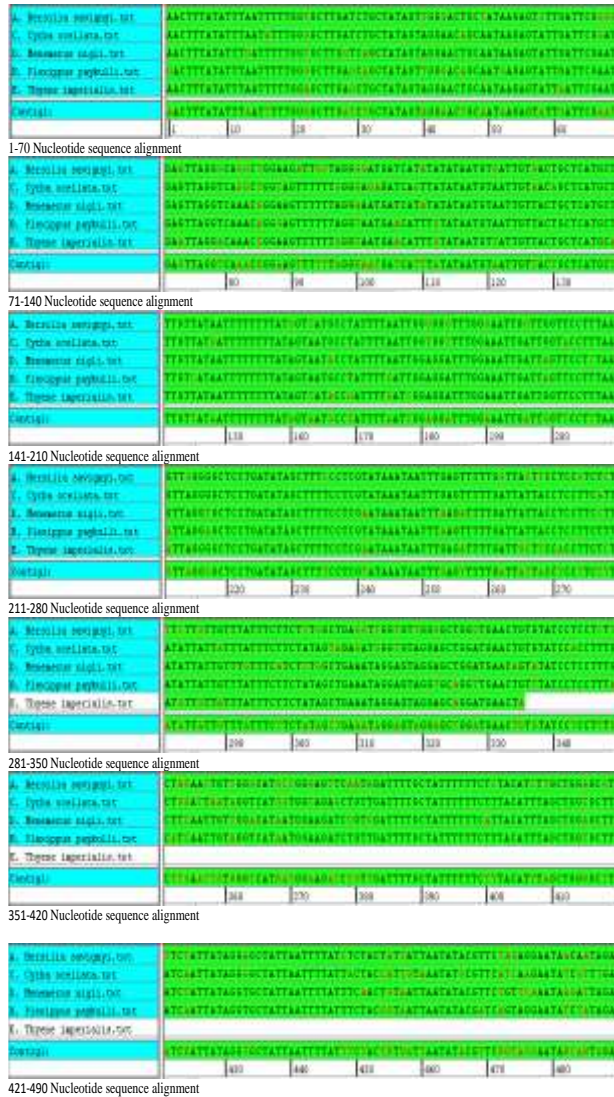
**Figs. 31–46:** *P. paykulli* Audouin 1826: 31–32, male and female habitus; 37, ventral view palp; 38, retrolateral view; 39, ventral view of female epigyne; 40, dorsal view. 33–34, *M. nigli* Wesolowska & Freudenschuss 2012 male and female habitus; 41, ventral view palp; 42, retrolateral view; 43, ventral view epigyne; 44, dorsal view; 35–36, *T. imperialis* Rossi, 1846 male and female habitus; 45, palp ventral view; 46, retrolateral view; 47, habitat



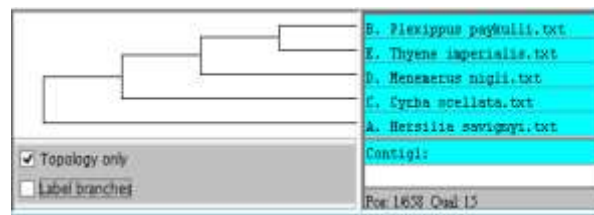
**Fig. 47:** Collection sites of the spiders

*Euophrys ocellata* Kroneberg, 1875: 48, pl. 5, f. 35 (Df).  
*C. ocellata* Wanless 1984b: 455, f. 7A–F, 8A–G, 18A–C (mf, S).  
*C. ocellata* Davies & Zabka, 1989: 194, pl. 6 (mf).  
**Sample id:** ICUP-MS-0017  
**Process id:** SPKPK017-20  
**Match BIN:** BOLD:ACR0186

**Match Process:** GBMIN114626-17.  
**Material studied:** Pakistan, KP, Talash Dir Lower, Barcharay hill, (34.71646E, 71.91679N, 1193m), 1♂3♀, 05.vi.2019, M. Sajid.  
**Measurement:** BL. 4.5–5.5, CL. 2–2.5, CW.1.3–1.6, AL. 3.1–3.4, AW. 1.3–1.7. Legs formula: 4132. Legs length: Leg I. 5.6, Leg II. 4.96, Leg III. 4.98, Leg IV. 6.5.



**Fig. 48:** Nucleotide sequence alignments result of ----- gene by codon code aligner version 8.02 of the . Lucas, 1836, *P. paykulli* Audouin 1826, *C. ocellata* Kroneberg, 1875, *M. nigli* Wesolowska & Freudenschuss 2012 and *T. imperialis* Rossi, 1846



**Fig. 49:** Homology Dendrogram showing genetic similarity among *P. paykulli* Audouin 1826, *C. ocellata* Kroneberg, 1875, *M. nigli* Wesolowska & Freudenschuss 2012 and *T. imperialis* Rossi, 1846, while deviation of *H. savignyi* Lucas, 1836 as a different family

**Distribution:** Eastern Africa to India and Indonesia, Caucasus to Central Asia and China. Introduced to Australia (Queensland) (WSC, 2020), while present study extends its ranges to Pakistan as well.

***Plexippus paykulli* Audouin 1826**  
Figs. (31, 32, 37, 38, 39, 40)

*Attus paykullii* Audouin 1826: 409, pl. 7, f. 22 (Dm).

*P. paykulli* Prószyński 2017b: 15, f. 4H, 5H (mf).

**Sample id:** ICUP-MS-0001

**Process id:** SPKPK001-20

**Match BIN:** BOLD:AAO2151

**Match Process:** MASPD018-10

**Material studied:** Pakistan, KP, Dir Lower, **Ajo hills**, (34.76228E, 71.92470N, 1170m), 3♂2♀, 21.vii. 2018, M. Sajid, Pakistan, **Nagri Payeen**, Dir Lower, KP, (34.74702E, 71.93470N, 1001m), 3♂2♀, 25.v. 2018, M. Sajid, Pakistan, **Nagri Payeen**, Dir Lower, KP, (34.74260E, 71.93947N, 995m), 1♂1♀, 01.vi. 2018, M. Sajid, Pakistan, KP, **Goro hill**, Dir Lower, (34.44704E, 71.94288N, 784m), 1♀ 1♂, 2.ix.2018, M. Sajid, Pakistan, KP, **Kamranay hill**, Dir Lower, (34.78704E, 71.94208N, 884m), 1♀ 1♂, 7.iv.2019, M. Sajid.

**Comments:** This species has previous record from Pakistan (World Spider Catalog 2020). The present study confirms its existence from the Northern parts of Pakistan as well. It reveals that this species has a wide range of distribution.

**Menemerus nigli Wesolowska & Freudenschuss 2012**

*M. nigli* Ali *et al.*, 2018: 6, f. 5–13 (m, Df).

Figs. (33, 34, 41, 42, 43, 44).

**Sample id:** ICUP-MS-0086

**Process id:** SPKPK086-20

**Match BIN:** BOLD:AAQ0156

**Match Process:** MTSPD151-15

**Material studied:** Pakistan, **Ajo hills**, Dir Lower, KP, (34.76208E, 71.92670N, 1180 m), 1♀, 24.vii. 2018, M. Sajid, Pakistan, **Nagri Payeen**, Dir Lower, KP, (34.74708E, 71.92470N, 1005 m), 3♂2♀, 19.vii. 2018, M. Sajid, Pakistan, **Nagri Payeen**, Dir Lower, KP, (34.74621E, 71.93470N, 1009m), 3♂3♀, 19.vii.2018, M. Sajid. Pakistan, Khyber Pakhtunkhwa (KP), Talash Dir Lower, **Manjawaro toot shah** (34.734781E, 71.94682N, 1084m), 2♂ 12.viii.2018, M. Sajid.

**Comments:** This species was reported (only male species) from Turbat, Balochistan, Pakistan for the 1<sup>st</sup> time (Wesolowska and Freudenschuss 2012). Existence of the species was confirmed and the female specimen was also reported for the 1<sup>st</sup> time from Karak, Khyber Pakhtunkhwa, Pakistan by (Ali *et al.* 2018). Present study extends the distribution of the species to Northern areas of Pakistan and 1<sup>st</sup> report for District Dir Lower.

**Distribution:** Pakistan, India, Brazil, Thailand and Sri Lanka

**Thyene imperialis Rossi 1846**

Figs. (35, 36, 45, 46, 47, 48, 49)

*Attus imperialis* Rossi 1846: 12 (Dm).

*T. imperialis* Prószyński 2017b: 33, f. 15F–H, 18B (mf).

**Sample id:** ICUP-MS-0010

**Process id:** SPKPK010-20

**Match BIN:** BOLD:AAO2153

**Match Process:** MASPD561-10

**Material studied:** Pakistan, Khyber Pakhtunkhwa (KP), Talash Dir Lower, **Manjawaro top hill** (34.73471E, 71.94699N, 1080m), 4♂ 5♀, 14.vii.2018; Pakistan, KP, **Dir Lower**, Nagri Bala, (34.77111E, 071.95526N, 1374m), 1♀ 1♂ 18 vii, 2018, Two males (MP-296) and two females (MP-294), Pakistan, **Ajo hills**, Dir Lower, KP, (34.76208E, 71.92670N, 1180m), 2♀ 2♂, 21.vii. 2018; Pakistan, KP, Talash Dir Lower, **Barcharay (Sabonai) hill**, (34.71666E,

71.91689N, 1190m), 1♂, 17.v.2018; Pakistan, KP, **Goro hill**, Dir Lower, (34.44709E, 71.49288N, 764m), 1♀ 1♂, 9.vii.2018, all coll. M. Sajid.

**Comments:** *T. imperialis* existence is confirmed by (Logunov and Ponomarev 2020). Present study reveals that the species has wide range of distribution and confirms its existence for the first time from Dir Lower.

**Distribution:** Afghanistan, Algeria, Balearic Islands, Bosnia and Herzegovina, Canary Islands, China, Chios, Corfu, Corse, Crete, Dalmatia, Egypt, Greece, India, Indonesia, Iran, Israel, Italy, Kenya, Krakatau, Lao, Libya, Malaysia, Morocco, Myanmar, Pakistan, Portugal, Rhodes, Russia, Sardinia, Saudi Arabia, Sicily, Spain, Sudan, Syria, Tajikistan, Thailand, Tinos, Tunisia, Turkey, Turkmenistan, United Arab Emirates, Yemen (World Spider Catalog 2020).

## Discussion

A total six species are reported from Dir Lower Pakistan. Three out of these six species are reported for the first time from Pakistan while the other three species from the study area. One species was from family Ganaphosidae, one from family Hersiliidae and four species were from family Salticidae. *M. dives* reported for the first. Earlier only three species of the genus reported are *M. dives concolor* Caporiacco 1935b, *M. lenzi* Bösenberg 1899, *M. pulcherrima* Caporiacco 1935b. This study increased the species fauna of *Micaria* from 3 to 4 in Pakistan. From nearby countries like Afghanistan 3 species, Iran 8 species and China 16 species and from India 3 species of the genus are reported (World Spider Catalog 2020).

*H. savignyi* is reported for the 1<sup>st</sup> time from Dir Lower. The genus is also new to the country. This is the only species of the genus reported from Pakistan. Surrounding countries like India and China each has 7 reported species of the genus, Iran with only one reported species and Afghanistan with no reported species (World Spider Catalog 2020).

As compared to other families, family Salticidae is described well from Pakistan but very few studies done with comparison to surrounding countries specially India and China. *C. ocellata* Kroneberg 1875 is reported for the 1<sup>st</sup> time from Pakistan. Also, the genus has doubtful existence in Pakistan (World Spider Catalog 2020). This study ensures the presence of the genus in Pakistan and increase the number of species of the family by one. Previously 69 species of the family were reported from Pakistan (Sajid *et al.* 2020). With addition of this new record the Salticid number reached to 70 species. Nearby countries like India and China have a well explored data about the family. From China 516 species from India 353, Iran with 103 reported species are far away in diversity exploration of the family from Pakistan. From Afghanistan 59 species are reported till now (World Spider Catalog 2020; Metzner 2020). To increase the biodiversity of Pakistan further study on exploration is must. This is the first study that provides illustrations for the species.

## Acknowledgments

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## Author Contribution

MS, MZ and AB planned the experiments, Identification and sample collection. MTK, MS and SA interpreted the results, MK and MS, R made the write original, editing, and made illustrations. All authors commented on the manuscript, reviewed drafts of the paper, and approved the final draft

## Conflict of Interest

The authors declare no conflict of interest

## Data Availability

Data reported in this study will be available on a fair request to the corresponding author

## Ethics Approval

Not applicable in present work

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