


First population estimates of the vulnerable southeast Iberian endemic tiger beetle *Cephalota (Taenidia) deserticoloides*

JOSÉ HERRERA-RUSSERT,^{1,2}  ALEJANDRO LÓPEZ-LÓPEZ,^{2,3,4} JOSÉ SERRANO,² ADOLFO CORDERO-RIVERA⁵ and JOSÉ GALIÁN^{2,6} ¹Department of Zoology and Physical Anthropology, University of Murcia, Murcia, Spain, ²Department of Zoology and Physical Anthropology, University of Murcia, Murcia, Spain, ³Departamento de Agroquímica y Medio Ambiente, Universidad Miguel Hernández, Alicante, Spain, ⁴Department of Invertebrate Evolution, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland, ⁵Universidade de Vigo, ECOEVO Lab, E.E. Forestal, Campus A Xunqueira, Pontevedra, Spain and ⁶Arthropotech SL, Arthropod Biotechnology, Nave Apícola - Granja Veterinaria UMU, Carretera de Guadalupe, Murcia, Spain

Abstract. 1. The tiger beetle *Cephalota deserticoloides* is a species found in a few localised sites in south-eastern Iberia, where it is a highly specialised inhabitant of the arid saline steppe habitat. Although regarded as vulnerable, very little is known about the actual population dynamics and degree of endangerment of this taxon, which may be worse than previously reported.

2. In this work, mark-recapture estimates of total population size are presented for one of the main known populations of *C. deserticoloides*. Additionally, some further remarks on seasonality and co-occurring tiger beetle species are made.

3. At the seasonal peak of adult activity, the area under consideration holds a relatively dense tiger beetle population with around 865 simultaneously active adult beetles, which is numerically comparable to those of other endangered cicindelids. These results will help assess the conservation status of *C. deserticoloides* and set the stage for more long-term efforts, which are clearly needed to analyse population viability, and the priority of *C. deserticoloides* and its habitat as targets for protective measures.

4. Our observations indicate that a reconsideration of the current International Union for Conservation of Nature (IUCN) listing of *C. deserticoloides* is strongly justified, from its current status as ‘vulnerable’ to a new listing as ‘endangered’.

Key words. Cicindelid, mark-recapture, population, tiger beetles.

Introduction

Tiger beetles are a widely distributed family (Pearson, 1988; Pearson & Cassola, 1992) that includes over 2600 species (Pearson & Cassola, 2005), spanning a high variety of habitats. Tiger beetles have received more attention than other insect taxa in fields such as natural history, population dynamics, community ecology, diversity pattern analysis and the taxonomy of certain groups within the family (Galián *et al.*, 1990; Knisley & Schultz, 1997; Cardoso & Vogler, 2005; López-López

et al., 2016). This amount of data, together with their high habitat specialisation, strengthens the case for their use as biological indicators (Rodríguez *et al.*, 1998; Pearson & Vogler, 2001).

The genus *Cephalota*, to which 25 species have been ascribed worldwide (Wiesner, 1992), is a halophile group that was likely already found populating the coasts of the Tethys Sea 35 million years ago (Hieke, 1983). This genus is distributed throughout the Mediterranean coast and deep into central Asia, with the species *C. littorea* (Forskål, 1775) and *C. vonderdeckeni* Gebert, 1992 reaching as far south as the Sudan and Somalia (Gebert, 1999; Werner, 2000). The current distribution patterns of most extant species were in all likelihood shaped by the formation of the Mediterranean Sea, resulting in vicariance events across

Correspondence: José Herrera-Russert. Email: jhr_a@msn.com

north–south and east–west axes (Herrera-Russert *et al.*, 2020). In this context, *Cephalota deserticoloides* (Codina, 1931) is an Iberian endemic that has been postulated to be a vicariant taxon of *C. deserticola* (Falder, 1836), a Eurasian species (Diogo *et al.*, 1999). A recent phylogenetic analysis of the genus *Cephalota* shows that this taxon has a marked phylogenetic singularity with no extant close relatives (Herrera-Russert *et al.*, 2020). Several species, such as *C. deserticoloides* and *C. dulcinea* (López, de la Rosa & Baena, 2006), have extremely restricted natural distributions (López *et al.*, 2006; Rodríguez-Flores *et al.*, 2016), which makes them potential candidates for wildlife conservation plans; as recognised by the inclusion of *C. deserticoloides* in the Spanish Red Book of Endangered Invertebrates (Lencina & Serrano, 2011).

Cephalota deserticoloides (Fig. 1b) is solely recorded from a few sites in southeastern Spain (Lencina & Serrano, 2011; Serrano, 2013), located in a sublittoral narrow strip running from the surroundings of Elche (province of Alicante) to the vicinity of Alhama de Murcia (province of Murcia). This area is almost entirely flat and corresponds to a littoral and sublittoral sedimentary basin containing gypsum and marl soils that was alternately emerged or covered by the Mediterranean Sea during the Neogene (del Ramo Jiménez & Guillén Mondéjar, 2009). In particular, *C. deserticoloides* is found in patches of saline steppe soils with halophile vegetation (Fig. 1a). These sites have been greatly reduced and disturbed by drainage, desalination, and transformation into agricultural land since the 1970s. More recently, a large number of these sites has been altered by rubbish dumps and the growth of urban areas and industrial complexes, with seven of them outright extirpated in the province of Alicante. As a consequence, tiger beetle populations have been greatly reduced in number and size of occupied sites, which currently amount to three well-known locations, making *C. deserticoloides* a clear example of an endangered species (Diogo *et al.*, 1999; Lencina & Serrano, 2011). These three sites are one precarious and likely nonviable population in San Isidro de Albaterra, Alicante; a population in a highly degraded environment in Totana, Murcia, and the only well preserved and likely fully viable population in the protected

natural space of Rambla Salada, Murcia (Lencina & Serrano, 2011).

In spite of this dire situation, no serious characterisation of population size and seasonal fluctuation has been performed up to this date. However, such efforts have been made with closely related Iberian species such as *C. littorea* and *C. hispanica* (Gory, 1833) using mark-recapture methodologies (Serrano, 1990) in their Portuguese populations, as well as with *C. dulcinea* in central Spain (Polidori *et al.*, 2020) and less labour-intensive methods like index counting have been employed to obtain similar estimates for North American species of conservation interest, such as *Cicindela dorsalis* Say, 1817, *C. albissima* Rumpff, 1962 and *C. puritana* Horn, 1871 (Gowan & Knisley, 2014; Knisley *et al.*, 2014). *C. deserticoloides*, together with all of the other temperate-climate *Cephalota* species, exhibits a typical summer species life cycle (Serrano, 1990; Pearson & Vogler, 2001) involving an overwintering and likely multivoltine larval stage, and a unimodal phenological peak in the early to high summer, which quickly tapers off towards early July for early species such as *C. dulcinea* and *C. deserticoloides*, and in August for other, later species such as *C. circumdata* and *C. littorea* (Serrano, 1990; Rodríguez-Flores *et al.*, 2016).

The aim of this article is to provide initial estimates of the population size of *C. deserticoloides* in the site where beetle population has been less disturbed, the Humedal del Ajauque y Rambla Salada (Alicante, Spain), that may set the stage for the assessment of its precise status and long-term population tendencies through further studies and monitoring efforts. In addition, we also expect to gain insights into the possible interactions among coexisting tiger beetle species within the site.

Materials and methods

Area of study

The Rambla Salada natural space is, for the most of its extension, a narrow creek with well-delimited vegetation zones.

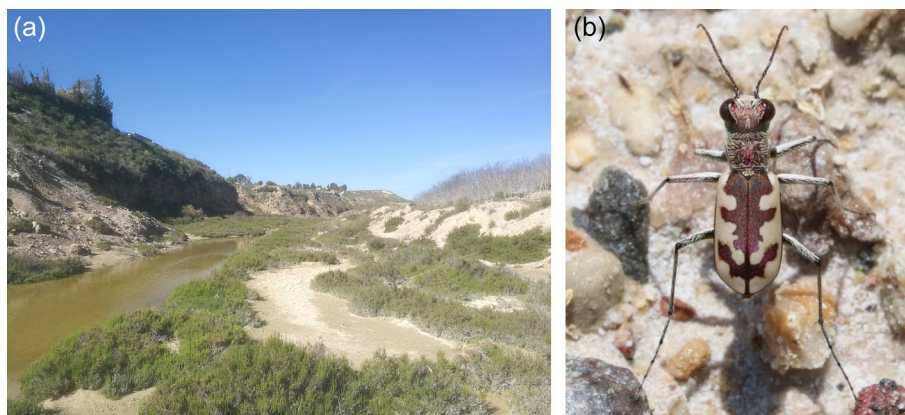


Figure 1. (a) Typical *C. deserticoloides* saline steppe habitat along Rambla Salada creek; (b) Habitus of the adult *C. deserticoloides*. [Color figure can be viewed at wileyonlinelibrary.com]

Cephalota deserticoloides inhabits the low, flat, and yearlong moist *Sarcocornia*-dominated clay strip between the very near approaches of the creek and the *Limonium* and *Tamarix* bush-dominated arid saline steppe (*Tamarix* rarely reaches tree size here) that starts abruptly with a sufficient ground-level increase. This strip of rather well-defined habitat is usually not wider than 10 m on each side of the waterline, often being much smaller and even disrupted due the effects of the growth of dense *Phragmites australis* (Cav) Trin ex Steud, 1841 and *Arundo donax* Linnaeus, 1753 formations, which have been brought about by a relatively recent decrease in salinity levels (Esteve Selma *et al.*, 1995).

Rambla Salada is included in the protected site 'Wetland of Ajauque and Rambla Salada' of the province of Murcia. The site covers 1632 ha distributed across the towns of Abanilla, Fortuna, Santomera and Molina de Segura. It was declared as a Zone of Special Protection for Birds in 2000. Rambla Salada is located at the bottom part of the drainage basin of the whole protected area, close to the tail of the reservoir of Santomera.

A patch of Rambla Salada where *Cephalota deserticoloides* occurs was selected on the basis of previous explorations of the stream searching for high beetle activity and because of

easy access from a nearby road (Fig. 2a). This site is known as Los Baños (38.116403 N, -1.089106 W). Suitable patches occupied by tiger beetles occurred for up to 2.4 km upstream, where another site was visually inspected for the duration of the study. This second site is known as Los Periquitos.

Mark-recapture study

We observed local moderate to high beetle densities from late May to late October, with an activity peak in the warmest hours of the day. Twelve sets of two pitfall traps were initially deployed in late May across a 150 m strip where individuals of *C. deserticoloides* were frequently observed (Fig. 2b). After concluding that a sufficiently large number of beetles were being captured, we decided to use this approach, which ensured optimal survival and sustained volume of captures in each trapping occasion. Beetles seemed most abundant from early June to mid-July, so the mark-recapture study was conducted over four trapping occasions between 12 and 21 June 2017.

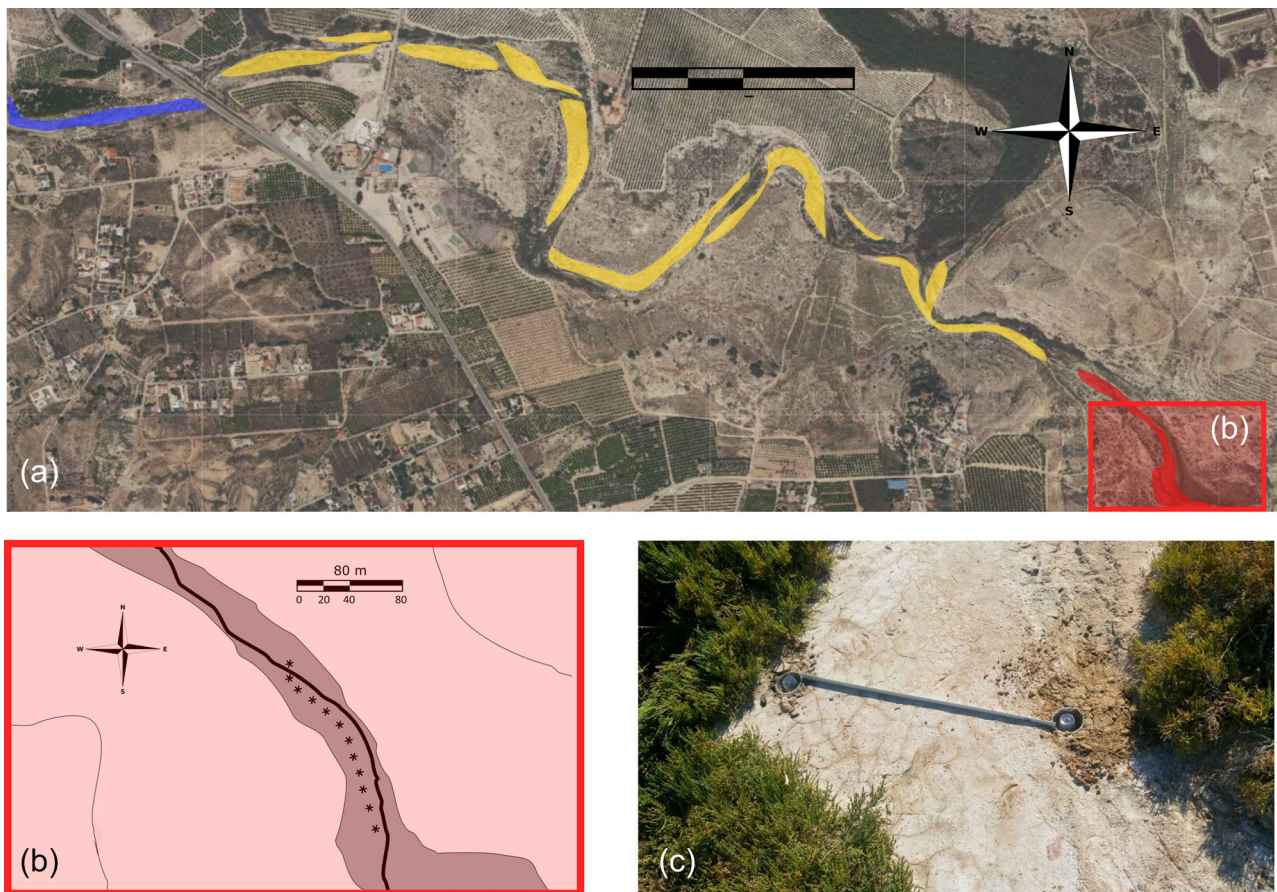


Figure 2. (a) Area along Rambla Salada including visually inspected Los Periquitos site (blue), Los Baños site (red, highlighted), where mark-recapture took place, and a further area where estimation was inferred (yellow); (b) Mark-recapture setup in Los Baños site, with individual trap positions (asterisks) and *C. deserticoloides* habitat (shaded); (c) 1 of the 12 pitfall barrier traps. [Color figure can be viewed at wileyonlinelibrary.com]

The specific need to capture the beetles along a narrow corridor of habitat was addressed by arranging the traps in pairs, each at either end of a 1 m long and 5 cm high aluminium barrier placed perpendicular to the stream and to the strip of beetle habitat (Hansen & New, 2005) (Fig. 2c). An inverted plastic cup (\varnothing 12 cm) was placed into each trap to retain the living insects (Taboada *et al.*, 2012), and traps were covered with plastic plates when not in use. Traps were inspected every 2 days during a 9-day sampling period from 12 June to 21 2017.

Daily work involved an early visit between 8:00 and 9:00, before any significant adult beetle activity, during which each trap was uncovered and cups put in place. Traps were then revisited at 18:00–20:30, during which time all captured tiger beetles were recorded, new ones were given an individual mark and all were released. The protarsi of adult tiger beetles are sexually dimorphic, bearing a dense pubescence in males but remaining bare in females, and this was used as the main cue for external sex assignment of each beetle. Traps were then covered for the night. Tiger beetles were marked on the prothorax with a customised numbering system using tempera paint. A double code system was partially used (Hagler & Jackson, 2001), by painting a coloured mark on the elytral tips of each individual to ensure that no marks were lost or repeated.

To estimate population size (N), we compared two approaches for closed populations (Amstrup *et al.*, 2010): the Schnabel index (Schnabel, 1938), which is an extension of the Lincoln–Petersen method to more than two sampling occasions, and Huggins closed capture models, which are a maximum likelihood estimator (MLE) which allows for the inclusion of covariates (Chao & Huggins, 2010). Population closure could be assumed given the short-time intervals involved (Krebs, 1999), and the short-term dynamics of tiger beetle populations, which have received similar treatments in other studies (Lovari *et al.*, 1992; Polidori *et al.*, 2020).

The Schnabel index was computed as follows:

$$N = \frac{\sum_{i=1}^m MiCi}{\sum_{i=1}^m Ri}$$

where C_i refers to the number of individuals captured during visit i , R_i refers to the number of previously marked individuals caught on visit i and M_i is the total number of marked individuals just before the i th site visit. Confidence intervals were calculated with R based on the methods outlined by Krebs (1999).

Population estimates were additionally derived using the MLE approach in program MARK (White and Burnham, 1999), in which we considered four models: $M(\cdot)$, which assumed capture probability p constant, $M(t)$, which assumed p to be affected by a time effect, $M(b)$, where p was subject to a behavioural effect and $M(t,b)$, in which p was affected by both time and behaviour. Equivalent models considering sex group effects were then added to the analysis. We considered the model with the lowest AICc (corrected Akaike information criterion) value for our comparative estimation of N (Burnham & Anderson, 2004).

Our study site of Los Baños is a relatively well-delimited area of high beetle density, but a continuous strip of variably widening or constrained potential habitat also exists up to the inspected area of Los Periquitos, where we also observed high beetle densities. We inspected the full extent of these two sites and the intervening area of potential habitat with Google Maps and Sig-Pac (sigpac.magrama.es) in order to hypothesise other potential hotspots of *C. deserticoloides* occurrence.

Results

A total of 123 *C. deserticoloides* specimens was sampled in the studied area of Los Baños. *Cephalota littorea* was also occasionally trapped ($N = 3$), while nearby clusters of *Myriochile melancholica* (Fabricius, 1798) were also noted. However, these three species appeared to be spatially well segregated, and despite their immediate presence, an overwhelming majority (over 95%) of captured tiger beetles were *C. deserticoloides* due to our trap placement. In total, 95 individual beetles were recaptured at least once. Individual beetles seemed to be caught at random positions in different trapping occasions, with only seven beetles having been captured at least two times at the same trap and no neighbouring effect observed.

The Schnabel index revealed a population estimate of 173 individuals (95% LCI of 128 and UCI 255 individuals). The Mt model (time varying p) was the most appropriate model based on AICc in the MLE approach. Delta AICc to the next model was also high enough (>10) for us to derive our population estimates exclusively from this model, which predicted a total of 173 individuals: 108 males (LCI 102, UCI 121) and 65 females (LCI 60, UCI 74). Although our estimates show a higher number of trapped males (Fig. 3), the resulting selection of model $M(t)$ implied that group effects had a weak influence on capture probabilities.

The trapping area in Los Baños comprised roughly 2000 m² where high densities of adult *C. deserticoloides* adults were observed, excluding any area where beetles were absent or rare, such as dense plant cover, water surfaces and the muddy shorelines, which were instead occupied by *C. littorea* and *Myriochile melancholica*. The inspected area of los Periquitos comprised around 1800 m², and we also carefully considered around 8200 m² in the intervening area as likely to support high beetle densities. Converting our estimates to beetle density and applying it to this combined area, we thus suggest an estimate of 865 tiger beetles clustered around highly favourable strips of habitat along the whole area considered in our study.

Discussion

Population size of Cephalota deserticoloides and conservation policy

The estimated population size of *Cephalota deserticoloides* falls well within the range suggested for other endangered tiger beetle species, far from the hundreds of thousands or millions of adults typical of a fully viable tiger beetle population as



Figure 3. (a) Total counts of individual male and female *C. deserticoloides* captured at each numbered trap along sampling area; (b) same counts at each of the two pitfall traps of each numbered trapping unit (I represents left side; D represents right, closer to stream). [Color figure can be viewed at wileyonlinelibrary.com]

suggested by Willey and Perkins (2007). For instance, the population of the endangered *C. puritana* is known to consist of six metapopulations inhabiting patches of 1100–9200 active adults, while *C. dorsalis* is known from several tens of locations populated by 7000–12 000 adult animals (Knisley & Schultz, 1997). With our estimated adult abundance remaining in the hundreds in its best preserved locality, *C. deserticoloides* thus seems closer to *C. ohlone* Freitag and Cavanaugh, 1993, a critically imperilled species for which exhaustive population assessment results have been made available (Arnold & Knisley, 2018).

C. deserticoloides has been collected from three localities in the last decades (Lencina & Serrano, 2011), to which two new but very small occurrences of a few individuals may soon be added (López-López, pers. com.). While our extended population estimates are based on strict extrapolation of high quality habitat with high beetle densities and thus are likely rather optimistic, they are informative enough to reflect the true scale of *C. deserticoloides* adult population size. Rambla Salada is the best-preserved site for the species, and we do not expect any other of its known locations to produce higher population estimates (Lencina & Serrano, 2011). The similarly threatened cicindelids *Cicindela puritana* and *C. ohlone* are, respectively, ‘endangered’ and ‘critically endangered’ under IUCN criteria, yet *Cephalota deserticoloides* retains its ‘vulnerable’ status exclusively on the basis of its high degree of endemism (Lencina & Serrano, 2011). We therefore suggest a reassessment for *C. deserticoloides* and its placement on the IUCN list as an endangered species.

Proper population assessment can only occur under long-term monitoring, and we suggest that this is an urgently needed measure for the preservation of the remaining *C. deserticoloides* populations. The historical fall in salinity and the fluctuation of

water line levels have enabled both extensive invasion by *Phragmites australis*, as well as the drying up of previously favourable habitats for *C. deserticoloides* in different areas, and these factors have likely already had a negative effect on the species. These phenomena, which have not been investigated in relation to *C. deserticoloides* population trends, may currently continue to increase habitat fragmentation and hence place further strain on existing populations.

Interactions of Cephalota deserticoloides with other species

Aside from *C. deserticoloides*, two other tiger beetle species were encountered, *Cephalota littorea* and *Myriochile melancholica*. The study areas of Los Baños and Los Periquitos visually exhibited similar densities of adult tiger beetle activity. However, our almost exclusive trapping of *C. deserticoloides* despite the presence of these other species suggests limited ecological overlap between the adult beetles.

Many tiger beetle species tend to occur in more or less discrete clusters of individuals (Simon-Reising *et al.*, 1996), a pattern that we observed for all three species in the area. *C. littorea* and *M. melancholica* were restricted to wet or flooded areas, while *Cephalota deserticoloides* was only observed on humid but not damp unvegetated soil, which may reflect its ecologically specialised character (Diogo *et al.*, 1999). Temporal patterns involved peak abundances from late May to late June for *C. deserticoloides* while *C. littorea* and *M. melancholica* peaked in early July, when *C. deserticoloides* was already on the decline. Taken together, these observations suggest a pattern of niche partitioning, common among tiger beetle assemblages (Brosius & Higley, 2013), which should be further explored to

understand the biology of *C. deserticoloides* as a possible specialist species of the arid saline steppe. We observed several instances of *C. deserticoloides* feeding on *Cataglyphis* ants and more occasionally on the woodlouse *Porcellio ornatus* Milne-Edwards, 1840. The observations of Polidori *et al.* (2020) on the closely related *C. dulcinea* suggest that this species may also be mainly myrmecophagous, a behaviour that seems to entail fitness costs which may also apply to *C. deserticoloides*.

Conclusion

It seems clear that a stronger conservation policy should be implemented to preserve the future of this tiger beetle, one that is tightly associated with the conservation of water bodies in saline-steppe areas of southeast Spain, which are known to harbour a rich biodiversity (Sánchez-Fernández *et al.*, 2006; Zafrilla Requena *et al.*, 2010; Millán *et al.*, 2011). Within this policy, new studies should be conducted in the other sites where the species occurs: the salt marsh of La Alcanara, a site included in the protected natural area of 'Saladares del Guadalentín' near Alhama de Murcia, and in San Isidro de Albaterra; in the latter, there is no conservation policy concerning biodiversity.

The study of the population size of *C. deserticoloides* may benefit from a more extensive mark-recapture effort to assess individual mobility and to establish whether there are discrete boundaries between populations as those described in some North American species. Although genetic distance is another approach to this problem, the mark-recapture procedure may offer a better view of the effect of vegetation change and salinity variations that are taking place rapidly.

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Ethical Statement

The applicable legislation was applied during manipulation of specimens and habitat involved in this study. Habitat was not disturbed or altered in any significant way, and no animals were harmed in obtaining our data.

Consent

All authors had a significant part in the production of this article and consent to its publication.

Conflict of Interest

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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