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Formicidae Faunal Succession on Buried Rabbit Carcass: Implications for Forensic Entomology

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ABSTRACT

Background: Buried carrion can help by forensic investigation cases in determining the post-burial-interval (PBI), movement of the body, or hiding of the crime. The current study aims to determine the succession of ant species on rabbit carcasses buried in an outdoor habitat of the King Saud University, Western Riyadh, Saudi Arabia.

Methods: In the present study, twelve rabbits were buried at 20 cm and twelve at 40 cm in two different periods. A day prior to the burial, a total of 24 holes measuring 60 × 30 cm were excavated using a short-handled spade. After 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 days, one cadaver from each depth was unearthed.

Results: We have found 8 species of Formicidae in this study. four types at a depth of 20 cm and two types only at a depth of 40 cm in the first period, while two types were found at a depth of 20 cm and one type only at 40 cm in the second period of the experiment. In the first period of the experiment, *Cataglyphis holgerseni* (Formicinae) and *Cardiocondyla* sp. (Myrmicinae) were the most prevalent species at depths of 20 cm and 40 cm, respectively; however, in the exposed carcasses, *Messor ebeninus* (Myrmicinae) predominated in both study periods. While no species dominated the buried bodies in the second phase, the numbers were very few in the second period on buried carcasses. In this study, many Formicidae species that correlate to different stages of corpse decomposition that were previously unknown from buried bodies in Saudi Arabia were recorded. Therefore, it is important and may have consequences for medicolegal issues.

Key words: Ants, Buried carcass, Forensic Entomology, Succession.

INTRODUCTION

Ants (Hymenoptera: Formicidae) are one of the most common insects in terrestrial habitats. They participate actively in the trophic system and are very helpful in the ecological decomposition process. They predate other arthropods that approach corpses (Catts, 1990) or eat directly on corpses (Bonacci *et al*., 2011; Ramón and Donoso, 2015).

Diptera are the most common order of insects discovered in a decomposing carcass and are used to estimate the minimum postmortem interval (PMImin) since they are the first insects to find and lay eggs on the cadaver (Smith, 1986). Coleoptera, the second-most common order in carcasses, are present during all stages of decomposition but particularly in the final stages (Mise *et al*., 2007). Hymenoptera is the third most abundant order of insects in corpses, primarily ants (Dos Santos, 2019), generally taking place during all stages of breakdown (Paula *et al*., 2016).

Physical barriers such as burial, bodies enclosed in containers, drums, or suitcases and various others may hamper insect activity. The time taken by insects to colonies the carrion placed above ground would be less than that of the carrion buried underground. Often, it has been seen that criminals bury the dead in shallow graves in haste, as digging requires much time and effort. Shallow grave entomofauna may also vary from deep graves, as some insects that can reach the thin soil cover may not locate the corpse buried deep (Sharif and Qamar, 2021).

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Ants have a direct impact on the decomposition process, demonstrate that they can exploit animal carcasses as food sources and impede the activity of other insects that are crucial to forensic investigations, such as flies and beetles (Singh *et al*., 2020). On corpses, it can behave in a variety of ways. Necrophagous ants, like *Camponotus sericeiventris*, rip out pieces of the carcass and carry them to their nest, speeding up the breakdown process and causing injuries to the body that end up serving as a doorway for Diptera larvae to access wet areas, facilitating their feeding. Predator ant species *Camponotus* (*Tanaemyrmex*) sp. can delay the decomposition process because they carry a great number of eggs of flies to their nest for later feeding, thus interfering with decomposition (Paula *et al.*, 2016). In any situation,

ants are a crucial species for forensic professionals because they can hasten or slow down the process of decomposition and inflict injuries that may look like they were sustained before death, leading to false conclusions (Patel, 1994).

Newer studies have stated that forensic hints provided by ants affect the rate of remains decomposition (Campobasso *et al*., 2009; Chin *et al*., 2009), postmortem artifacts removal by ants (Heath and Byard, 2014) and ant species composition affect the position of carcasses (Chen *et al.*, 2014).

To our knowledge, in the Kingdom of Saudi Arabia, no research was conducted in an attempt to identify the types of ants on buried corpses. In order to identify species and successions of ants, we carried out this investigation in the city of Riyad, Saudi Arabia, using rabbit carcasses as an animal model.

MATERIALS AND METHODS

Study site

The research was done in Riyadh at King Saud University, 43°24 N, 46°36 E, on a 175 m by 250 m plot of ground containing acacia trees and several common wild herbs.

Study time

The study was conducted in two periods: the first period from late February to late May 2021 and the second period from September 5, 2021, to January 4, 2022.

Environment data

For the duration of the experiment, air temperature and relative humidity were recorded hourly using a Lascar EL-USB-2 data logger. and an EM50G data logger (Ecotone, Gdynia, Poland) was used for soil and was programmed to record the temperature and humidity once each hour throughout the experiment (Table 1).

Carrion

The Animal Use Committee of Al-Imam Muhammad Ibn Saud Islamic University in Riyadh, Saudi Arabia, approved the use of rabbits as the research animal for this study. Project number: 35-2021. 50 live, mature rabbits (*Oryctolagus cuniculus* L.) were obtained locally, weighing between 1400 - 2200 g. A day prior to the burial, a total of 24 holes measuring 60 × 30 cm were excavated using a short-handled spade. These holes were dug at depths of both 20 and 40 cm. The pits were positioned at least 10 meters apart from each other on all sides. On the morning of the burial, chloroform was employed to euthanize all the rabbits. The rabbits were positioned on a section of chicken wire measuring 30 × 60, which had holes with a diameter of 25 mm. This arrangement served the dual purpose of facilitating easier removal and preventing scavenging by other animals. 24 rabbits were buried in each study period and the exposed carcass was placed in robust steel 2-cm mesh cages with a layer of wire screening, each measuring 60 × 50 × 30 cm^3.

Examination procedure and taxonomic identification

The grave was opened and sampled every 10 days for 120 days (four months) in both periods for the collection and observation of insects. Ants were collected from the exposed soil at the bottom of the hole and the layers of soil cover, in addition to the body of the rabbit, by handpicking with forceps. Then placed in Eppendorf tubes with 70% ethanol. The Formicidae fauna was identified at species level when possible with the aid of the identification keys of Collingwood (1985). Also, to confirm the identification of the species, we referred to experts in the Insect Museum, College of Food and Agricultural Sciences, King Saud University. Specialists were consulted and comparisons were made with the standards of the Entomological Reference Collection.

RESULTS AND DISCUSSION

In total, eight types of ants were recorded in our study. In buried bodies, four types were recorded at a depth of 20 cm and two types only at a depth of 40 cm in the first period, while two types were found at a depth of 20 cm and one type only at 40 cm in the second period of the experiment. As for the exposed carcasses, seven types were recorded

Table 1: Mean daily temperature and humidity of ambient air for 120 days.

Day	Mean temperature °C		Mean humidity %	
	First period	Second period	First period	Second period
0-10	18	34.1	46	12.10
11-20	20	33.5	45.5	13
21-30	17.5	32.4	50	13.7
31-40	23	31.3	35.5	13.8
41-50	26	29.1	22	21.7
51-60	26.5	27.1	23	20.4
61-70	27	23.3	12.5	26.9
71-80	30	22.8	25	32.8
81-90	30	19.4	16.5	29.7
91-100	31	17.3	20	31.5
101-110	33	19.4	18	33.3
111-120	38	15.6	11.5	58.6

in the first period and six types in the second period (Table 2 and Fig 1).

These species follow three subfamilies: Myrmicinae, Formicinae and Ponerinae. Formicinae were prevalent in buried carcasses, while Myrmicinae were prevalent in exposed carcasses (Fig 2). *Cataglyphis holgerseni* (Formicinae) is the most abundant species at a depth of 20 cm and *Cardiocondyla* sp. (Myrmicinae) at a depth of 40 cm in the first period, while the numbers were very few in the second period of the experiment, but in the exposed carcasses, the species *Messor ebeninus* (Myrmicinae) was dominant in both study periods (Fig 3).

To the best of our knowledge, this is the first study of its kind to investigate ant fauna in buried carcasses and the resultant forensic implications. Previous work carried out in three different habitats (agricultural, desert and urban) in the city of Riyadh, Saudi Arabia, presented only Formicidae fauna associated with exposed carcasses (Mashaly *et al.*, 2018) and two habitats that were ecologically differentiated (Al-Mekhlafi *et al.*, 2021).

Carcass decomposition time differed significantly between the two experimental periods in exposed bodies, the decomposition time of which was considerably extended to 30 days in the First period and 17 days in the second period, explaining the highest relative abundance and richness of ant species in those carcasses since these carcasses were exposed longer to the action of these insects. In the study of Paula *et al.* (2016), they discovered that pig carcasses exposed longer also attracted a greater variety of species. while bodies buried at both depths (20 and 40) reached the dry stage after 60 days of burial in the two study periods.

The first appearance of ants in buried corpses was in the stage of decay, as the species Camponotus aegyptiacus appeared in the second excavation (20 days), then the species *Cataglyphis arenarius* on the 30th day and the species *Cataglyphis holgerseni* on the 40th day. The dry phase began at the sixth excavation (60 days) and the species *C. holgerseni* and *Cataglyphis arenarius* were recorded on the 70th day and the two species *Messor*

Table 2: Species of ants collected from buried and surface carcasses.

family	Genus and species	First period			Second period		
		soil surface	20 cm	40 cm	soil surface	20 cm	40 cm
Myrmicinae	*Monomorium niloticum*	√	--	--	√	--	--
	Messor ebeninus	√	√	--	√	--	--
	Cardiocondyla sp.	√	--	√	--	--	--
Formicinae	*Cataglyphis arenarius*	√	√	√	√	√	√
	Camponotus fellah	√	--	--	√	--	--
	Cataglyphis holgerseni	√	√	--	√	--	--
	Camponotus aegyptiacus	--	√	--	√	√	--
Ponerinae	*Brachyponera sennaarensis*	√	--	--			

Presence (√) or absence (--).

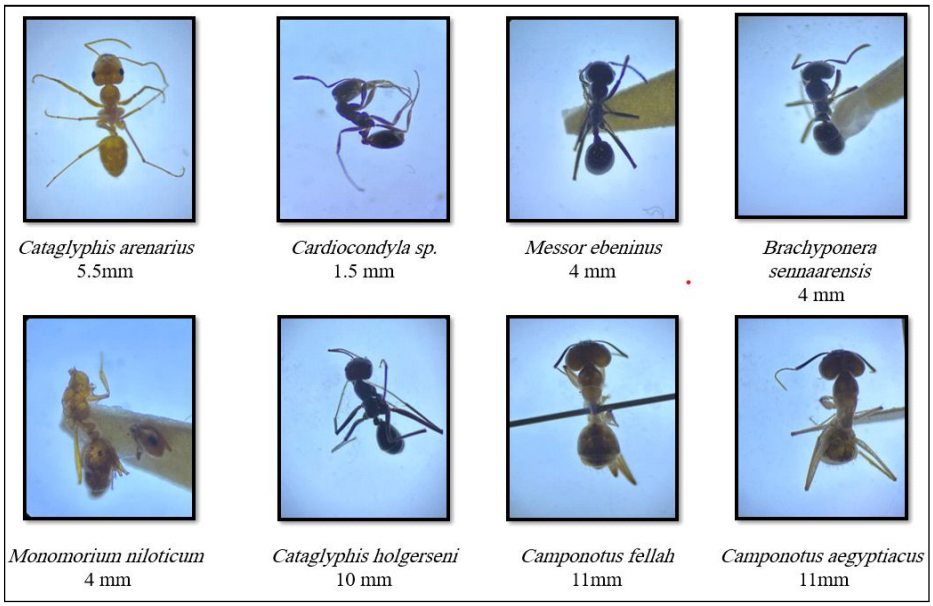

Fig 1: Ants collected during sampling.

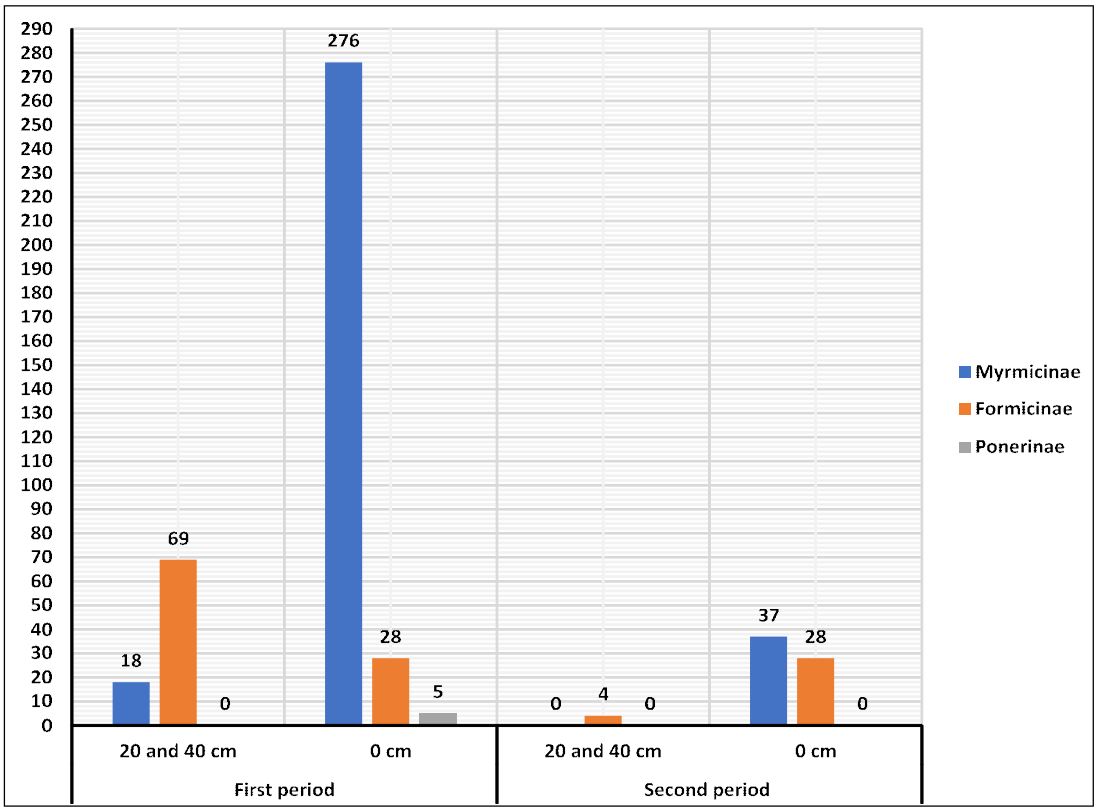

Fig 2: Abundance of ant families in carcasses at different depths 0, 20 and 40 cm in the two study periods.

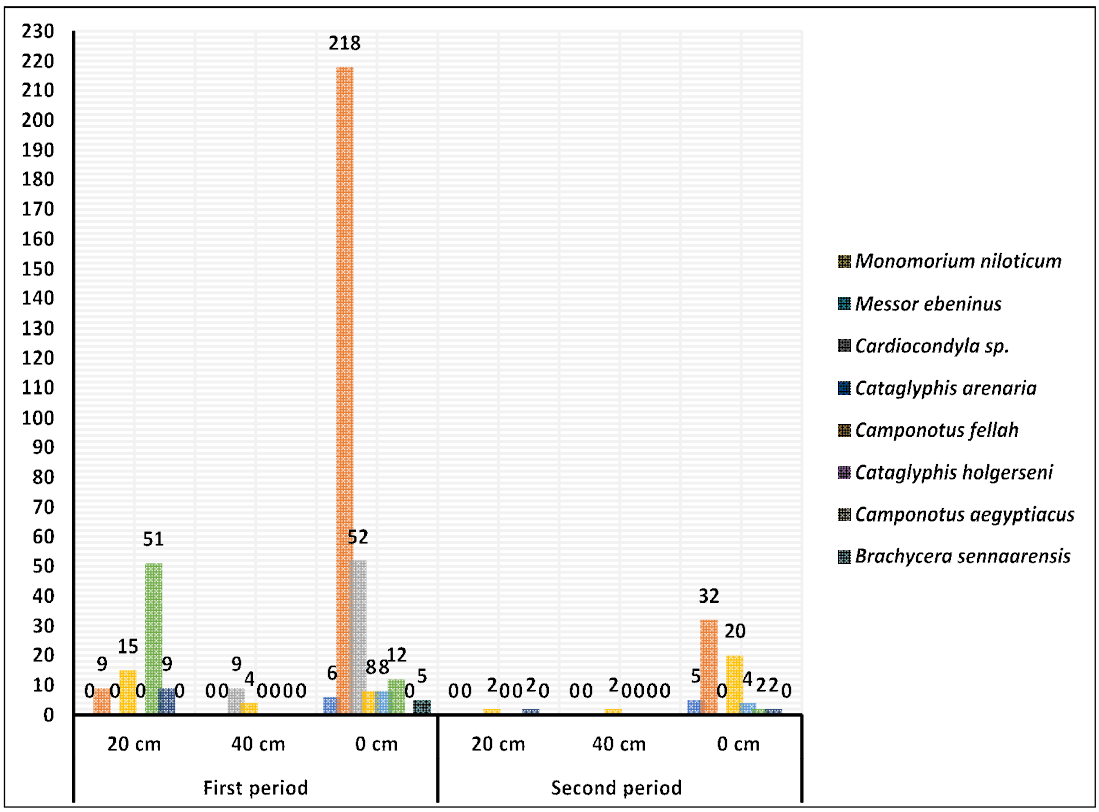

Fig 3: Abundance of ant species in carcasses at different depths 0, 20 and 40 cm in the two study periods.

Table 3: Absolute number of distinct Formicidae species in exposed and buried carcasses at various stages of decomposition

Subfamily	Genus and species	Depth/cm	Buried carcasses											Exposed carcasses			
			10	20	30	40	50	60	70	80	90	100	110-120	F:1-2 S: 1	3-7 2-3	8-12 4-7	13-30 8-17
			Bloated	Decay				Dry						Fresh	Bloated	Decay	Dry
Myrmicinae	*Monomorium*	20	-	-	-	-	-	-	-	-	-	-	-	-	F(6)	-	S(5)
	niloticum	40	-	-	-	-	-	-	-	-	-	-	-	-			
	Messor	20	-	-	-	-	-	-	-	-	F(9)	-	-	-	F(14)	F(133)	(71)
	ebeninus	40	-	-	-	-	-	-	-	-	-	-	-	-	S(6)	S(2)	S(24)
	Cardiocondyla	20	-	-	-	-	-	-	-	-	-	-	-	-	F(11)	F(19)	F(22)
	sp.	40	-	-	-	-	-	-	-	-	F(9)	-	-	-			
Formicinae	*Cataglyphis*	20	S(2)	-	F(15)	-	-	-	-	-	-	-	-	-	F(8)	-	-
	arenarius	40	-	-		S(2)	-	-	F(4)	-	-	-	-	-		S(13)	S(7)
	Camponotus	20	-	-	-	-	-	-	-	-	-	-	-	-	F(5)	-	F(3)
	fellah	40	-	-	-	-	-	-	-	-	-	-	-	-	S(2)	-	S(2)
	Cataglyphis	20	-	-	-	F(31)	-	F(20)	-	-	-	-	-	-	F(5)	F(3)	F(4)
	holgerseni	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S(2)
	Camponotus	20	-	F(9)	-	-	-	-	-	-	-	-	-	-	-	-	-
	aegyptiacus			S(2)													S(2)
		40	-	-	-	-	-	-	-	-	-	-	-	-			
Ponerinae	*Brachyponera*	20	-	-	-	-	-	-	-	-	-	-	-	-	F(5)	-	-
	sennaarensis	40	-	-	-	-	-	-	-	-	-	-	-	-			

F: First period, S: Second period.

ebeninus and *Cardiocondyla* sp. on the 90th day. As for the second period, the first appearance was in the Bloated stage of the type *C. arenarius*, then the type *C. aegyptiacus* in the decay stage and finally, *C. arenarius* reappeared on the 40th day and for the rest of the experiment period, no species was found (Table 3). As for the exposed carcasses, most of the species appeared in the three stages of decomposition (Bloated, decay and dry), except for *C. aegyptiacus*, which was found in the dry stage only and *Brachyponera sennaarensis* in the Bloated stage (Table 3).

M. niloticum appeared on exposed carcasses only and this species has not been recorded on cadavers before, but other species of the genus Monomorium have been recorded in previous studies; for example, *M. floricola* was found on rats in Malaysia (Singh *et al.*, 2020), *M. minimum* on pigs in Mississippi, USA (Goddard *et al*., 2012) and in Egypt (Hamdy *et al*., 2022) recorded *M. carbonarium*, *M. lipenyi*, *M. niloticus* and *M. salmonmis* on the carcasses of rabbits and guinea pigs.

M. ebeninus was one of the most abundant species and was present at all stages in both periods of the experiment in both surface and buried carrions at a depth of 20 cm. The genus *Messor* was observed on buried carrion collected on pigs buried in Italy (Bonacci *et al.,* 2021) and South Africa (Botham, 2016) and on superficial carcasses in Saudi Arabia (Al-Khalifa *et al*., 2021; Al-Qahtni *et al*., 2021).

The genus *Cardiocondyla* was collected only in the first period at a depth of 40 cm and on the exposed bodies at all stages of decomposition except the fresh and to our knowledge, this genus has not been recorded in the studies of forensic entomology in KSA, while many other studies have reported its association with buried (Botham, 2016) and surface carcasses (Chen *et al*., 2014; Leong *et al.*, 2019; Souza, 2020).

C. arenarius and *C. holgerseni* were collected in our current study in both periods and on Exposed and buried bodies in both depths. This genus was observed in Iran on a trap consisting of livestock, poultry and fish tissues (Tüzün *et al*., 2010); it was also recorded on the carcasses of rabbits buried in Iraq (Albushabaa, 2016); and in KSA on human corpses (Al-Khalifa *et al*., 2021; Al-Qahtni *et al*., 2021). Diet flexibility is a feature many ant species possess and can lead to drastic changes such as those of *B. sennaarensis,* which has been documented as a granivore species and can shift to a carnivorous diet depending on the particular nature of environmental conditions (Lachaud and Déjean, 1994). This is confirmed by our current study, where this species was found on rabbit carcasses in the first period of the study. It was also collected from exposed carcasses of Impala in South Africa (Braack, 1986).

Camponotus is a common ant genus found on corpses. It has been investigated by many authors and different species were associated with surface carcasses (Haddadi *et al*., 2019; Odo and Iloba, 2020; Souza, 2020) and buried remains (Bala and Kaur, 2015; Bonacci *et al*., 2021; Botham, 2016). This is consistent with our study, where the species *C. fellah* was found on superficial carrion only and the species *C. aegyptiacus* appeared on buried corpses at a depth of 20 cm and aboveground. (Viana *et al*., 2022) alone recorded 10 species of this genus on the carcasses of exposed pigs in Brazil. Souza *et al*. (2008) documented *C. rufipes* preying on eggs and larvae of flies in the bulging stage, *Camponotus sp.* Was observed manifesting similar behaviour by predating on *Calliphorid* larvae in our current study (Fig 4). Silahuddin *et al.* (2015) also observed *C. gigas* as predators of adult *Ch. megacephala*.

Fig. 4: Formicinae ants preying immature of dipterans

CONCLUSION

More research is needed in buried forensic entomology, particularly in Saudi Arabia where there is a dearth of information. Additionally, more thorough research must be done to create a global database on the succession of insects from various ecosystems with applications in the forensic and ecological disciplines. In the area of buried forensic entomology, the current work offers fundamental knowledge. We do, however, draw the conclusion that some insects can colonize buried carcasses, which calls for additional investigation and study in this area.

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Authors contribution

O. Al-Zahrani and F.A. Al-Mekhlafi designed the study, F.A. Al-Mekhlafi, M.S. Al-Khalifa and M.S. Al-Khalifa conducted data analyses and wote the manuscript. OA performed field experiments. F.A. Al-Mekhlafi, A.H. Al-Qahtni and M.S. Al-Khalifa writing the manuscript and helped in conducted data analysis.

Ethical approval

The conducted research is not related to either human or animal use.

Data availability statement

All the data is available within the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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