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Mosquito fauna associated with *Aedes aegypti* (Diptera: Culicidae) in Yucatán State of southeastern México, and checklist with new records

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Abstract

As part of our routine surveillance for arboviruses transmitted by *Aedes (Stegomyia) aegypti* (L.) (Diptera: Culicidae) in Yucatán State, México, we regularly monitor the mosquito fauna that occurs in the region. In this study, immature and adult mosquitoes were collected at 60 study sites (including residential premises, churches, tourist areas, cemeteries, forested areas, and garbage dump sites) in Yucatán State from Jul 2014 to Dec 2015. In total, 4,062 larvae, 344 pupae, and 3,812 adults representing 7 genera and 27 species were collected. Three species had never before been identified in Yucatán State, and these are *Aedes (Ochlerotatus) euplocamus* Dyar & Knab, *Aedes (Protomacleaya) podographicus* Dyar & Knab, and *Culex (Culex) declarator* Dyar & Knab. The identification of these 3 species brings the current total of mosquito species recognized in Yucatán State to 52 (with 11 genera). Many of the mosquitoes collected in this study were temporally and spatially associated with *Ae. aegypti*.

Key Words: *Aedes euplocamus*; *Aedes podographicus*; *Culex declarator*

Resumen

Como parte de la vigilancia de arbovirus transmitidos por *Aedes (Stegomyia) aegypti* L., en el estado de Yucatán, México; regularmente monitoreamos la fauna de mosquitos presentes en la región. En este estudio, los estadios de inmaduras y adultos de mosquitos fueron colectados en 60 localidades en el estado de Yucatán durante julio de 2014 y diciembre de 2015. Un rango amplio de lugares urbanos y rurales fueron examinados, incluyendo casas, iglesias, áreas turísticas, cementerios, selva y un basurero municipal. Un total de 4,062 larvas, 344 pupas y 3,812 adultos fueron colectados, representando siete géneros y 27 especies. Tres especies fueron identificadas como nuevas para la fauna de mosquitos de Yucatán: *Aedes (Ochlerotatus) euplocamus* Dyar and Knab, *Aedes (Protomacleaya) podographicus* Dyar and Knab y *Culex (Culex) declarator* Dyar and Knab. Adicionando estas tres especies, actualmente hay 52 especies (con 11 géneros) de mosquitos en Yucatán. Muchos de los mosquitos colectados en el presente estudio fueron temporal y espacialmente asociados con *Ae. aegypti*.

Palabras Clave: *Aedes euplocamus*; *Aedes podographicus*; *Culex declarator*

Aedes (Stegomyia) aegypti (L.) (Diptera: Culicidae) is the main vector of dengue and chikungunya viruses, both of which have been associated with human disease in Yucatán State, México (García-Rejón et al. 2008; Diaz-Gonzalez et al. 2015). *Aedes aegypti* mosquitoes are common in Yucatán State, and larvae and pupae are often found inside disposable containers, buckets, tires, flower pots, vases, and storm-

water drains/catch basins where present (Winch et al. 1992; García-Rejón et al. 2011; Arana-Guardia et al. 2014). *Aedes aegypti* occurs in a diverse range of habitats including residential premises, vacant lots, parking lots, and cemeteries (Baak-Baak et al. 2014a,b).

Immatures and adults of *Ae. aegypti* usually cohabit with other mosquito species (Baak-Baak et al. 2014b). During surveillance activi-

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SAMPLING OF IMMATURE AND ADULT MOSQUITOES

Methods used to collect mosquitoes and the classification of container types have been described in previous studies (Nájera-Vázquez et al. 2004; García-Rejón et al. 2011). Nets, turkey basters, or pipettes were used to collect immatures from water-holding containers. Larvae and pupae were placed in plastic containers, labeled according to date, study site, and sample identification number, and transported to the Laboratorio de Arbovirología at Universidad Autónoma de Yucatán. A portion of the larvae were killed in hot water (60 °C); the remaining larvae were individually reared to obtain adults. Pupae were allowed to emerge as adults, and the adults were identified to species. Adult mosquitoes were collected using Centers for Disease Control and Prevention backpack-mounted aspirators. Stereomicroscopes and taxonomic keys were used to identify species (Carpenter & LaCasse 1955; Schick 1970; Arnell 1976; Clark-Gil & Darsie 1983; Ibáñez-Bernal & Martínez-Campos 1994; Darsie & Ward 2005).

SEARCH CHECKLIST OF MOSQUITO SPECIES OF YUCATÁN STATE

We carried out a literature review on mosquito species in Yucatán State in the following databases: Google Scholar, PubMed Health [National Center for Biotechnology Information at the National Library of Medicine], SciELO (Scientific Electronic Library Online), and Web of Science (Thompson Reuters). The search was conducted with the introduction of keywords such as Culicidae, mosquito fauna, mosquito surveillance, new records, mosquito distribution, Yucatán, and México.

Results

A summary of the mosquitoes collected in this study is in Table 1. We collected 8,218 specimens representing 7 genera and 27 species. These species represent ~52% of all mosquito species known to occur in Yucatán State (references can be seen in Table 2). Three species had never before been identified in Yucatán State, and these are *Aedes (Ochlerotatus) euplocamus* Dyar & Knab, *Aedes (Protomacleaya) podographicus* Dyar & Knab, and *Culex (Culex) declarator* Dyar & Knab. We collected 4,406 immatures (4,062 larvae and 344 pupae) in 220 water-holding containers or natural breeding sites. We also collected 3,812 adult mosquitoes. The most common species were *Ae. aegypti* and *Cx. quinquefasciatus* (Fig. 1).

BIOLOGICAL NOTES

We provide notes for the 3 newly reported species. Information on their localities and ecological information is also provided.

Aedes euplocamus occurs in Colombia, Peru, Venezuela, Costa Rica, El Salvador, Guatemala, Guyana, Honduras, Panama, and México (Arnell 1976; Burkett-Cadena et al. 2013; Rozo-Lopez & Mengual 2015). In México, this species has been identified in the states of Veracruz (Arnell 1976), Quintana Roo (Ortega-Morales et al. 2010), and Oaxaca (Bond et al. 2014). Arnell (1976) performed a complete review of this species group (*Aedes: Ochlerotatus: Scapularis*): Larvae and pupae have been collected from water covered in partial shade and in rock-

Table 1. Number of mosquito species registered in Yucatán State from Jul 2014 to Dec 2015.

#	Taxon	Adult			Immature		
		Female	Male	Habitat	Larva	Pupa	Breeding
1	<i>Ae. aegypti</i>	550	880	House	1,700	150	Disposable container, tree hole, tire, bucket
2	<i>Ae. cozumelensis</i>	17	5	Forest, black mangrove ^a	25	7	Vase, tire, rock hole
3	<i>Ae. fulvus</i>	6	—	House	—	—	—
4	<i>Ae. euplocamus</i> ^b	2	—	Forest	—	—	—
5	<i>Ae. scapularis</i>	3	—	Forest	7	—	Rock hole
6	<i>Ae. taeniorhynchus</i>	150	15	Forest, house, black mangrove ^a	50	8	Rock hole, temporary pool
7	<i>Ae. trivittatus</i>	30	—	Forest, house, church	8	4	Rock hole
8	<i>Ae. podographicus</i> ^b	3	—	Forest	21	5	Tree hole
9	<i>An. albimanus</i>	5	—	House	8	—	Temporary pool, artificial lake (aguada)
10	<i>An. pseudopuctipennis</i>	—	—	—	7	3	Cenote
11	<i>Cx. coronator</i>	—	—	—	720	25	Bucket, rock hole
12	<i>Cx. declarator</i> ^b	—	—	—	120	8	Tire, rock hole
13	<i>Cx. interrogator</i>	35	5	House, church	300	5	Tire, vase, bucket
14	<i>Cx. lactator</i>	—	—	—	211	9	Vase, bucket
15	<i>Cx. nigripalpus</i>	150	25	Church	250	30	Vase, bucket
16	<i>Cx. quinquefasciatus</i>	800	1,100	House, church	500	70	Disposable container, bucket, tire
17	<i>Cx. salinarius</i>	—	—	—	4	—	Tire
18	<i>Cx. tarsalis</i>	2	—	House	—	—	—
19	<i>Cx. thriambus</i>	2	—	House	—	—	—
20	<i>Cx. erraticus</i>	—	—	—	15	—	Temporary pool, artificial lake (aguada)
21	<i>Cx. iolambdis</i>	12	3	Forest, black mangrove ^a	11	3	Temporary pool
22	<i>Cx. pilosus</i>	—	—	—	5	—	Temporary pool
23	<i>Cx. corniger</i>	—	—	—	20	5	Bucket, rock hole
24	<i>Li. durhamii</i>	2	—	Church	60	8	Vase, disposable container, bucket
25	<i>Ps. ferox</i>	3	—	Forest, church	—	—	—
26	<i>Ur. lowii</i>	7	—	Forest	5	1	Temporary pool
27	<i>Toxorhynchites</i> spp.	—	—	—	15	3	Rock hole, bucket, tire

^aGarbage collection site near of black mangrove (*Avicennia germinans*).

^bNew records for Yucatán State.

(-): not found.

Table 2. Checklist of the mosquito species known from Yucatán State and new records. Classification according to Knight & Stone (1977). Previous occurrence records are abbreviated: MI: Martini (1935); MP: Martínez-Palacios (1952); VA: Vargas (1956); VA-MP: Vargas & Martínez-Palacios (1956); WI: Winch et al. (1992); IB: Ibáñez-Bernal & Martínez-Campos (1994); NV: Nájera-Vázquez et al. (2004); ZP: Zapata-Peniche et al. (2007); GR-1: García-Rejón et al. (2008); FA-9: Farfán-Ale et al. (2009); FA-0: Farfán-Ale et al. (2010); GR-2: García-Rejón et al. (2011), OM-1: Ortega-Morales et al. (2011); GR-3: García-Rejón et al. (2012); MS: Manrique-Saide et al. (2012); AG: Arana-Guardia et al. (2014); BB: Baak-Baak et al. (2014a); NR: never reported in Yucatán.

#	Taxon	Immature	Adult
1	<i>Aedes (Howardina) cozumelensis</i> Díaz Nájera	IB, GR-3, BB, present study	Present study
2	<i>Aedes (Stegomyia) aegypti</i> (Linnaeus)	WI, IB, NV, ZP, GR-2, MS, AG, BB, present study	GR-1, FA-1, FA-2, GR-2, present study
3	<i>Aedes (Ochlerotatus) angustivittatus</i> Dyar and Knab	NR	MI
4	<i>Aedes (Georgecraigius) epactius</i> Dyar and Knab	IB	NR
5	<i>Aedes (Ochlerotatus) euplocamus</i> Dyar and Knab ^a	NR	Present study
6	<i>Aedes (Ochlerotatus) fulvus</i> Wiedemann	NR	FA-2, present study
7	<i>Aedes (Ochlerotatus) infirmatus</i> Dyar and Knab	NR	FA-1
8	<i>Aedes (Ochlerotatus) scapularis</i> (Rondani)	IB, NV, ZP, present study	
9	<i>Aedes (Ochlerotatus) serratus</i> Theobald	NR	MI
10	<i>Aedes (Ochlerotatus) sollicitans</i> (Walker)	VA, IB, NV, ZP	NR
11	<i>Aedes (Ochlerotatus) taeniorhynchus</i> (Wiedemann)	VA, IB, NV, AG, BB, present study	MI, GR-1, FA-1, FA-2, GR-2, present study
12	<i>Aedes (Ochlerotatus) tortilis</i> Theobald	NR	MI
13	<i>Aedes (Ochlerotatus) trivittatus</i> (Coquillett)	BB, present study	GR-1, FA-1, FA-2, GR-2, present study
14	<i>Aedes (Protomacleaya) podographicus</i> Dyar and Knab ^a	Present study	Present study
15	<i>Anopheles (Nyssorhynchus) albimanus</i> Wiedemann	IB, NV, present study	VA-MP, FA-1, FA-2, present study
16	<i>Anopheles (Anopheles) apicimacula</i> Dyar and Knab	IB	VA-MP
17	<i>Anopheles (Anopheles) bradleyi</i> King	IB	VA-MP
18	<i>Anopheles (Anopheles) crucians</i> Wiedemann	IB	VA-MP, FA-1, FA-2
19	<i>Anopheles (Anopheles) pseudopuctipennis</i> Theobald	IB, present study	VA-MP, FA-1
20	<i>Anopheles (Anopheles) vestitipennis</i> Dyar and Knab	IB	VA-MP, FA-1, FA-2
21	<i>Culex (Culex) bidens</i> (Dyar)	IB, NV, ZP	
22	<i>Culex (Culex) coronator</i> s.l. Dyar and Knab	VA, WI, IB, NV, ZP, MS, AG, BB, present study	FA-1, FA-2
23	<i>Culex (Culex) declarator</i> Dyar and Knab ^a	Present study	NR
24	<i>Culex (Culex) interrogator</i> Dyar and Knab	IB, NV, ZP, MS, AG, BB, present study	FA-1, FA-2, GR-2, present study
25	<i>Culex (Phenacomyia) lactator</i> Dyar and Knab	ZP, AG, BB, present study	NR
26	<i>Culex (Culex) nigripalpus</i> Theobald	IB, NV, ZP, BB, present study	FA-2, present study
27	<i>Culex (Culex) quinquefasciatus</i> Say	VA, WI, IB, NV, ZP, MS, AG, BB, present study	MI, GR-1, FA-1, FA-2, present study
28	<i>Culex (Culex) restuans</i> Theobald	IB	NR
29	<i>Culex (Culex) salinarius</i> Coquillett	IB, ZP, MS, AG, BB, present study	NR
30	<i>Culex (Culex) stigmatosoma</i> Dyar	IB, OM-1	Present study
31	<i>Culex (Culex) tarsalis</i> Coquillett	IB, AG, BB	FA-1, present study
32	<i>Culex (Culex) thriambus</i> Dyar	IB, NV, ZP, MS, AG, BB, present study	NR
33	<i>Culex (Melanoconion) erraticus</i> (Dyar and Knab)	MP, VA, IB, present study	NR
34	<i>Culex (Melanoconion) iolambdis</i> Dyar	IB, present study	Present study
35	<i>Culex (Melanoconion) pilosus</i> (Dyar and Knab)	IB, present study	NR
36	<i>Culex (Phenacomyia) corniger</i> Theobald	IB, present study	Present study
37	<i>Haemagogus (Haemagogus) anastasionis</i> Dyar	IB, NV	NR
38	<i>Haemagogus (Haemagogus) equinus</i> Theobald	IB, NV, BB	NR
39	<i>Haemagogus (Haemagogus) mesodentatus</i> Komp & Kumm	IB, NV	FA-1, FA-2
40	<i>Limatus durhamii</i> Theobald	BB, present study	Present study
41	<i>Mansonia (Mansonia) titillans</i> (Walker)	IB	FA-1, FA-2
42	<i>Psorophora (Grabhamia) confinnis</i> s.s. (Lynch-Arribalzaga)	IB, NV	FA-1
43	<i>Psorophora (Janthinosoma) albipes</i> Theobald	NR	FA-1
44	<i>Psorophora (Janthinosoma) cyanescens</i> (Coquillett)	VA, IB	MI, FA-1, FA-2
45	<i>Psorophora (Janthinosoma) ferox</i> (von Humboldt)	IB	MI, FA-1, FA-2, present study
46	<i>Psorophora (Psorophora) ciliata</i> (Fabricius)	IB	FA-1
47	<i>Psorophora (Psorophora) howardii</i> Coquillett	NV	FA-1, FA-2
48	<i>Deinocerites cancer</i> Theobald		MI
49	<i>Uranotaenia (Uranotaenia) lowii</i> Theobald	VA, IB, present study	MI, FA-2, present study
50	<i>Toxorhynchites (Lynchiella) theobaldi</i> (Dyar and Knab) ^b	VA, ZP	NR
51	<i>Toxorhynchites</i> spp.	Present study	
52	<i>Wyeomyia (Wyeomyia) celaenocephala</i>	NR	MI

^aNew records.

^bCited by Vargas (1956) as *Megarhinus theobaldi* Dyar and Knab.

holes without vegetation. *Aedes euplocamus* often cohabits with *Ae. aegypti* and various *Culex* and *Uranotaenia* species (Arnell 1976). It is not considered to be a vector of pathogens of medical or veterinary

importance. In the present study, we collected 2 females of *Ae. euplocamus* in a forest in Xmatkuil (20.8501747°N, 89.6002139°W), which is a small town close to Mérida City.

The geographic distribution of *Ae. podographicus* encompasses Belize, Nicaragua, Panama, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, México, and Venezuela (Schick 1970). In México, *Ae. podographicus* has been reported in the states of Campeche (Schick 1970), Chiapas (Bond et al. 2014), Jalisco (Martini 1935), Nayarit (Schick 1970), Oaxaca (Martini 1935; Schick 1970), Quintana Roo (Ortega-Morales et al. 2010), Guerrero (Cortés-Guzmán et al. 2013), and Tamaulipas (Ortega-Morales et al. 2015). Larvae and pupae have commonly been collected from tree holes (Schick 1970). *Aedes podographicus* is not known to vector any pathogens of medical or veterinary importance. Although Martini (1935) and Ibáñez-Bernal & Martínez-Campos (1994) reported the presence of *Ae. terrens* in Yucatan, there have not been further reports of this particular mosquito's species in México (Zavortink 1972; Darsie 1996). Therefore, it is arguable that the above mentioned authors could have mistakenly identified the more common mosquito *Ae. podographicus*, which has extensively been reported in Mexican territories. In the present study, we collected 3 adult females of *Ae. podographicus* in a forest in Xmatkuil (20.8501747°N, 89.6002139°W). We also collected larvae and pupae of *Ae. podographicus* in a tree hole in Tizimin City (21.1169106°N, 88.1334789°W), in the east of Yucatán. Immatures of *Ae. aegypti* were also present in the same collections.

Culex declarator has a very wide distribution ranging from southern Texas through to México, Central America, and South America. Older literature, such as Carpenter & LaCasse (1955), has named this species *Cx. virgultus* Theobald. In México, *Cx. declarator* has been identified in the states of Quintana Roo (Ortega-Morales et al. 2010), Guerrero (Bond et al. 2014), and Tamaulipas (Ortega-Morales et al. 2015). The larvae are found in a variety of habitats, including rock pools, swamps, cement drains, rot cavities in trees, and coconut husks (Carpenter & LaCasse 1955). *Culex declarator* is of public health importance. St. Louis encephalitis virus was detected in *Cx. declarator* in Trinidad (Aitken et al. 1964) and Brazil (Monath et al. 1980). *Culex declarator* is also considered to be a potential vector of *Dirofilaria immitis*, dog heartworm (Labarthe et al. 1998). In the present study, immatures of *Cx. declarator* were collected in rock holes in a rural area in Ekmul (20.9501358°N, 89.3336006°W), Tixkokob, and from a tire at a trash collection site (21.2667072°N, 89.7577778°W) located near black man-groves in Progreso City.

CHECKLIST OF MOSQUITO SPECIES KNOWN FROM YUCATÁN

Between 1935 (Martini 1935) and 2014 (Arana-Guardia et al. 2014; Baak-Baak et al. 2014b), we found 17 works related to the record and distribution of mosquito species in Yucatán State, representing 11 genera and 49 species. The addition of 3 more species brings the species count to 52. The highest richness of culicid taxa belongs to *Aedes* (17 species), *Culex* (16 species), *Anopheles* (7 species), and *Psorophora* (6 species) (Table 2).

Discussion

Dengue virus is endemic in Yucatán State, and recently chikungunya virus has been associated with human morbidity in this region (García-Rejón et al. 2008; Diaz-Gonzalez et al. 2015, Cigarroa-Toledo et al. 2016). Both of these viruses are primarily vectored by *Ae. aegypti* (García-Rejón et al. 2008; Diaz-Gonzalez et al. 2015). For this reason, we routinely monitor the activity of *Ae. aegypti* and its associated viruses in Yucatán State. In the present study, we identified 27 mosquito species that occupy the same niche as *Ae. aegypti*, including 3 species never before reported in Yucatán State: *Ae. euplocamus*, *Ae. podographicus*, and *Cx. declarator*. Globally, the family Culicidae comprises

3,549 species distributed among 112 genera (Harbach 2013). It was recently reported that 217 to 239 species and 15 to 16 genera of mosquitoes have been documented in México (Bond et al. 2014).

In an entomological investigation performed by Ibáñez-Bernal & Martínez-Campos (1994), 36 species of mosquitoes were identified in Yucatán State. Additionally, Vargas (1956) reported 9, Nájera-Vázquez et al. (2004) reported 16, and Farfán-Ale et al. (2009, 2010) reported up to 20 species of mosquitoes in Yucatán State.

It should be noted that most studies on mosquitoes have been carried out in Mérida, because it is the largest city in Yucatán State and the incidence of dengue is high in this location (García-Rejón et al. 2008). Previous studies have shown that immature mosquitoes of various species coexist with immatures of *Ae. aegypti* in disposable containers, buckets, tires, and storm-water drains in Mérida (Winch et al. 1992; Zapata-Peniche et al. 2007; Ortega-Morales et al. 2011; Manrique-Saide et al. 2012; Arana-Guardia et al. 2014; Baak-Baak et al. 2014b). Adult mosquitoes of various species often coexist with adults of *Ae. aegypti* inside houses in Mérida (García-Rejón et al. 2008). Currently, there are 33 species of mosquitoes registered in Mérida City (Baak-Baak et al. 2014b).

At the state level, Tamaulipas and Quintana Roo have the highest richness of culicid taxa with 82 and 76 species, respectively (Ortega-Morales et al. 2010, 2015), followed by Chiapas with 54 species (Bond et al. 2014). As noted earlier, the revised total for Yucatán State is now 52 species. However, additional species could be present within the state but have remained undetected because we usually use trapping methods and focus on sites that allow for the collection of high numbers of *Ae. aegypti*. Other states where entomological investigations have recently been performed are Guerrero with 38 species, Oaxaca with 34 species (Bond et al. 2014), and Colima with 28 species (Espinoza-Gomez et al. 2013).

In the present study, many of mosquitoes identified are capable of serving as vectors of pathogens affecting humans or domestic animals in México. A notable absence is *Ae. (Stegomyia) albopictus* (Skuse), previously recorded in the Yucatán Peninsula (Salomón-Grajales et al. 2012) but not found in the present study. Martini (1935) reported the identification of *Anopheles intermedius* (Peryassu), but Vargas & Martínez-Palacios (1956) considered it to be a misclassification and suggested that *An. apicimacula* Dyar and Knab had been collected. Notably, we collected *An. albimanus* Wiedemann and *An. pseudopunctipennis*; these are the main vectors of the parasite that causes malaria in southeastern México (Martini 1935; Vargas & Martínez-Palacios 1956). Additionally, we recorded *Ae. taeniorhynchus*, which is a major vector of *D. immitis* (dog heartworm) in Yucatán State (Manrique-Saide et al. 2010).

In the historical literature, it appears that some species of mosquitoes in Yucatán State were erroneously reported. For example, Vargas (1956) apparently collected *Aedes (Ochlerotatus) punctor* (Kirby), but this species is not considered to occur in México (Darsie 1996). Additionally, Ibáñez-Bernal & Martínez-Campos (1994) reported immatures of *Aedes (Protomacleaya) triseriatus* (Say). Recently, Sánchez-Trinidad et al. (2014) revised the geographical distribution of this species and concluded that it is only distributed in northern and central México. It is currently unclear as to which *Toxorhynchites* species occur in Yucatán State. *Toxorhynchites (Lynchiella) theobaldi* (Dyar and Knab) was reported by Vargas (1956) and Zapata-Peniche et al. (2007). However, Zavortink & Chaverri (2009) examined the status of *Toxorhynchites* species by their distinctive tarsal markings from several collections and suggested that *Tx. moctezuma* (Dyar & Knab) and *Tx. hypoptes* (Knab) had been collected instead. Additionally, the Mosquitoes of Middle America Project makes no mention of these species occurring in México (Heinemann & Belkin 1977). For convenience, *Toxorhynchites* spe-

cies collected in our study were identified only to the genus level and will later be conclusively identified using molecular tools. We did not find mosquitoes of the genera *Haemagogus*, *Mansonia*, *Deinocerites*, and *Wyeomyia*, which have all previously been reported in Yucatán State. However, we provide valuable information on mosquito fauna temporally and spatially associated with *Ae. aegypti* in this region.

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