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Reproductive phenology of three species of *Gracilaria*: *G. blodgettii* Harvey, *G. vermiculophylla* (Ohmi) Papenfuss and *G. salicornia* (C. Agardh) Dawson (Gracilariales, Rhodophyta) from Okinawa, Ryukyu Islands, Japan

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Abstract—Reproductive phenology of three edible agarophytes of *Gracilaria*, *G. blodgettii* Harvey, *G. salicornia* (C. Agardh) Dawson and *G. vermiculophylla* (Ohmi) Papenfuss, were studied from February 2002 to January 2003 in Okinawa Is., southern Japan. The maturation period, ratio of each reproductive phase and seasonal changes in the fresh weights of these species were determined. The tropical species, *G. blodgettii*, appeared in December and decreased through June. The ratio between tetrasporophytes and gametophytes was almost even except for the end of maturation period. Meanwhile, the temperate species, *G. vermiculophylla*, was confirmed during December to March. The ratio of tetrasporophytes and gametophytes was almost 2 to 1, although tetrasporophytes was more frequent during February and March. On the other hand, another tropical species, *G. salicornia*, was found throughout the year, but the reproductive plants were found only between December and June. Spermatangial plant of this species was confirmed for the first time in Japan. *Congracilaria babae* Yamamoto, an adelphoparasite that grows on *G. salicornia*, was confirmed only during June. The dominance of the three species in winter and spring in Okinawa Is. suggests that their growth is strongly influenced by seawater temperature.

Key words: Okinawa, *Gracilaria*, Gracilariaceae, Gracilariales, Japan, phenology, Rhodophyta, seasonal changes

Introduction

The red algal genus *Gracilaria* (Gracilariaceae, Gracilariales) as well as the two genera, *Gracilariopsis* and *Hydropuntia*, has been regarded as a major source of the phycocolloid, agar, in the world (Abbott 1988, Critcheley 1993). They are also harvested for local food and are used as ingredients for soup and salad in a variety of East and Southeast Asian countries (Critcheley and Ohno 1998).

The Ryukyu Islands is an archipelago in the western Pacific Ocean that lies between the Japan mainland and Taiwan (Fig. 1). The species of Gracilariaceae are well represented in the shores of this archipelago, with sixteen species recognized for the region (Yoshida 1998, Terada et al. 2000, Terada and Ueno 2004, Terada and Shimada 2005). Some species that occur principally in Southeast Asian countries are also known from this island chain, which is believed to be their northern distributional limit (cf. *Gracilaria blodgettii* Harvey and *Gracilaria salicornia* (C. Agardh) Dawson, etc.). Meanwhile, *Gracilaria vermiculophylla* (Ohmi) Papenfuss

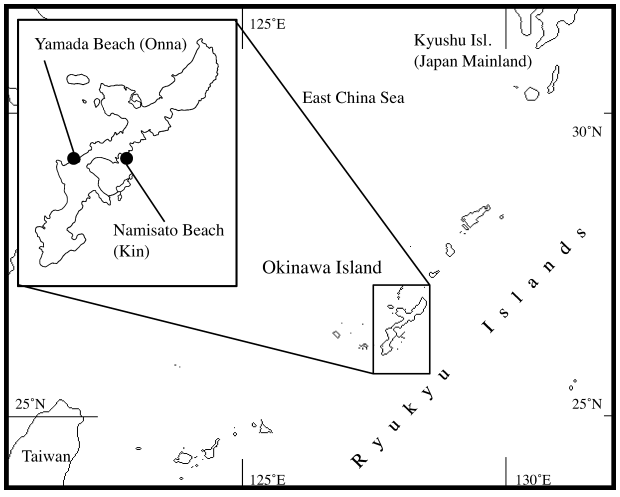


Fig. 1. Map showing Okinawa Island and adjacent waters including the study sites.

distributed principally in the temperate region of Japan is also found in these islands, which is their southern limit, suggesting that the flora of Gracilariaceae in this region can be regarded as the ecotone of tropical and temperate species.

In the Ryukyu Iss., some species of Gracilariaceae have been harvested from natural populations in coral lagoons for use as an ingredient of salads and for gracilarioid jelly. These foods are indispensable to local dietary culture in this region. Although *G. vermiculophylla* is also harvested in the mainland of Japan as well as *Gracilariopsis chorda* (Holmes) Ohmi or *Gracilaria parvispora* Abbott (=*Gracilaria bursa-pastoris* auct. japon, (Kim et al. 2008)), it is mainly used for the production of agar.

Ecological studies of Gracilariaceae have been reported from a variety of locations in the Pacific Ocean since 1970's (e.g., Holyle 1978, Nelson 1989, Kim et al. 1993, Luhan 1996. Pondevida and Hurtado-Ponce 1996), and they have contributed towards the elucidation of their biomass and their seasonal changes in relation to the life history. These studies have also contributed to establishing strategies to conserve the natural resource and the improvement of cultivation techniques.

Ecological studies have also been done in Japan regarding the Gracilariaceae such as *G. vermiculophylla* (Terada et al. 2000), *Gracilariopsis chorda* (Ohmi 1958) and *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik (Chirapart et al. 1995). In general, it is known that the mature gametophyte is hardly seen in natural populations of some species of *Gracilaria* and *Gracilariopsis*. In particular, Terada et al. (2000) reported that spermatangial plants of *G. vermiculophylla* of Hokkaido Island, northern Japan, appeared only in early summer (June). It is also known that the natural populations of *Gracilariopsis lemaneiformis* from Shikoku Is. and *Gracilariopsis chorda* var. *exilis* Yamamoto from Hokkaido Is. consist of sporophytes only (Chirapart et al. 1995, Yamamoto and Yamauchi 1996). These studies suggest that the life history of this group need further studies.

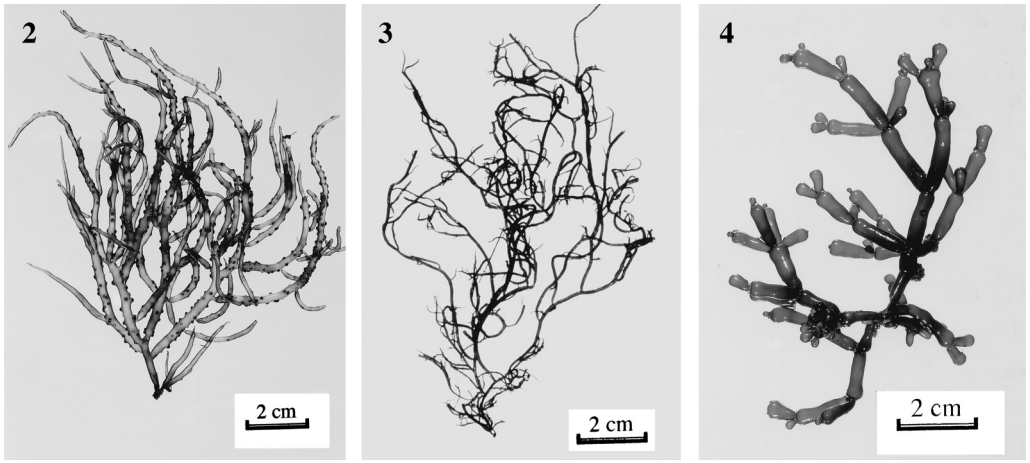
As there is insufficient knowledge of tropical species from the Ryukyu Iss. except for the study by Gerung et al. (1997) on *G. blodgettii*, we conducted field surveys to elucidate the seasonal changes of three edible *Gracilaria*, *G. blodgettii*, *G. salicornia* and *G. vermiculophylla* from Okinawa Is.

Materials and Methods

The study sites were located along the northwestern and southeastern coasts of Okinawa Is.: 1) Yamada Beach (26°26′12″N, 127°40′53″E), Onna Village; and 2) Namisato Beach (26°26′59″N, 127°56′37″E), Kin Town (Fig. 1). These two sites were subjected to moderate wave activity in the coral lagoon and a maximum diurnal tidal range of 2.0 m. *G. vermiculophylla* was collected from the former, while *G. blodgettii* and *G. salicornia* were collected from the latter (Figs. 2–4). Sampling was conducted seven times (Feb. 28, Mar. 28, Apr. 27, Jun. 22, Sep. 12, Dec. 23 and Jan. 9) from February 2002 to January 2003. Due to poor weather conditions, we could not complete survey for *G. salicornia* in February and March. At the each sampling-date, sixty- (*G. vermiculophylla*) and thirty-plants (*G. blodgettii* and *G. salicornia*, respectively) were randomly collected, and directly transported to the laboratory of Marine Botany, Kagoshima University.

Plants collected were washed with sterile seawater in the laboratory, and were examined for their reproductive status. In this study, we defined reproductive plants as those plants have either a spermatangial conceptacle, tetraporangium or cystocarp. Fresh weight of each plant was measured with an electronic scale (1212MP, Sartorius AG, Göttingen).

Surface seawater temperature and dissolved oxygen were measured at the study sites with a portable dissolved oxygen meter (Model 85, Yellow Spring Instruments Incorporated, Ohio), and light intensity at the surface of the seawater was measured with a light meter (510-03, Yokogawa Co., Tokyo). Voucher specimens are deposited in the Herbarium of Kagoshima University Museum, Kagoshima (*KAG*).



Figs. 2–4. Three *Gracilaria* species from Okinawa Island. 2: *G. blodgettii*, 3: *G. vermiculophylla*, 4: *G. salicornia*.

Results

Habitat characteristics and environmental conditions

Each *Gracilaria* species was found growing on pebbles or dead coral at the lower intertidal to sublittoral zone in the sandy bottom of a coral lagoon. Surface seawater temperatures at Namisato and Yamada Beach ranged from 19.1°C on February 21 to 32.1°C on June 22, and from 20.8°C on January 8 to 32.9°C on September 12, respectively (Table 1). Given that *Gracilaria* was growing inn the shallow waters of the near-shore up to 20 cm deep during the lowest tide, the surface seawater temperatures in these sites were influenced by direct sunlight and/or land temperature leading to relatively lower or higher water temperatures than that of offshore sites of the coral lagoon. During the study period, salinity was somewhat stable between 32.0 psu and 33.8 psu, suggesting that there was little influence of fresh water at the study site. Dissolved oxygen was also stable and ranged from 5.59 mg L^{-1} and 10.38 mg L^{-1}. Day length ranged from 10 h 27 min to 13 h 39 min. The highest light intensity on recorded during the surveys was 126.4 klux.

Table 1. Water temperature, salinity, dissolved oxygen, day length and light intensity at the study sites: Namisato Beach of Kin Town and Yamada Beach of Onna Village, both in Okinawa Island, Japan.

Namisato Beach, Kin

Date	W. T. (°C)	Salinity (psu)	DO (mg/L)	Day length	Light Intensity (Klx)
28-Feb	21.0	ND	ND	11:27	86.9
28-Mar	22.0	ND	ND	12:11	6.8
27-Apr	25.3	33.2	ND	12:55	6.0
22-Jun	32.1	33.7	5.59	13:39	125.9
12-Sep	31.3	32.3	6.79	12:18	111.1
23-Dec	24.6	32.0	8.93	10:21	21.7
9-Jan	19.1	32.2	10.38	10:27	103.4

Yamada Beach, Onna

Date	W. T. (°C)	Salinity (psu)	DO (mg/L)	Day length	Light Intensity (Klx)
28-Feb	21.7	ND	ND	11:27	86.8
28-Mar	22.5	ND	ND	12:11	61.3
26-Apr	23.6	33.8	7.68	12:55	10.5
21-Jun	32.0	33.4	8.29	13:39	126.4
12-Sep	32.9	33.5	6.47	12:18	87.6
23-Dec	23.4	33.8	7.64	10:21	ND
8-Jan	20.8	33.6	8.96	10:26	7.2

Gracilaria blodgettii

G. blodgettii was confirmed from December to June, and the maximum frequency of vegetative plants occurred in December and was 21%. The ratio of tetrasporophytes to gametophytes was almost similar in proportion, although there were some differences in timing of their monthly peaks (Fig. 5). Tetrasporophytes occurred frequently in June (66%), but during other months this frequency ranged from 28% (December) to 43% (January). Spermatangial plants occurred at rates less than 33% in April. In particular, no mature spermatangial plant was found in June despite the presence of cystocarpic plants. Cystocarpic plants occurred throughout the presented period from 18% (June) to 38% (December). The ratios of total vegetative plants, tetrasporophytes, spermatangial and cystocarpic plants throughout the year were : 11 : 44 : 17 : 28.

Fresh weights during each reproductive phase showed almost similar seasonality with some differences in size and peaks (Fig. 6). Fresh weights of spermatangial plants increased from December and showed a peak in February at 2.68±1.03 g. Thereafter, it decreased and disappeared by June. Cystocarpic plant showed similar trends as the spermatangial plants with the maximum size 4.40±1.36 g (February). Tetrasporophytes also showed similar seasonality as the cystocarpic plant, and maximum size was observed during February at 4.49±2.61 g.

Gracilaria vermiculophylla

G. vermiculophylla was found during December to March, and was absent during April to September (Fig. 5). Reproductive plants were confirmed in December at a rate of 14%, and was abundant in February (96%) and March (100%). The ratio of tetrasporophytes to gametophytes was represented similarly, and tetrasporophytes showed a high occurrence in February (43%) and March (41%). In December and February, the ratios of spermatangial to cystocarpic plants were almost even. However, the proportion of spermatangial plants decreased in March to 13%. The ratios of total vegetative plants, tetrasporophytes, spermatangial and cystocarpic plants throughout the year were: 11:44:17:28.

Fresh weight during each reproductive phase showed almost similar seasonality with some differences in size and period (Fig. 6). Fresh weight of spermatangial plants showed a peak in February at 0.89±0.03 g, then decreased and disappeared by April. Cystocarpic plants also showed similar seasonality as the spermatangial plants with a maximum size of 2.07±1.04 g (February). Tetrasporophytes appeared and showed a peak in February (3.07±0.76 g). This size slightly decreased in March, and disappeared by April.

Gracilaria salicornia

G. salicornia was found during the study period, however, the plants were rare in September (Fig. 5). Reproductive plants were confirmed from December to June. Although

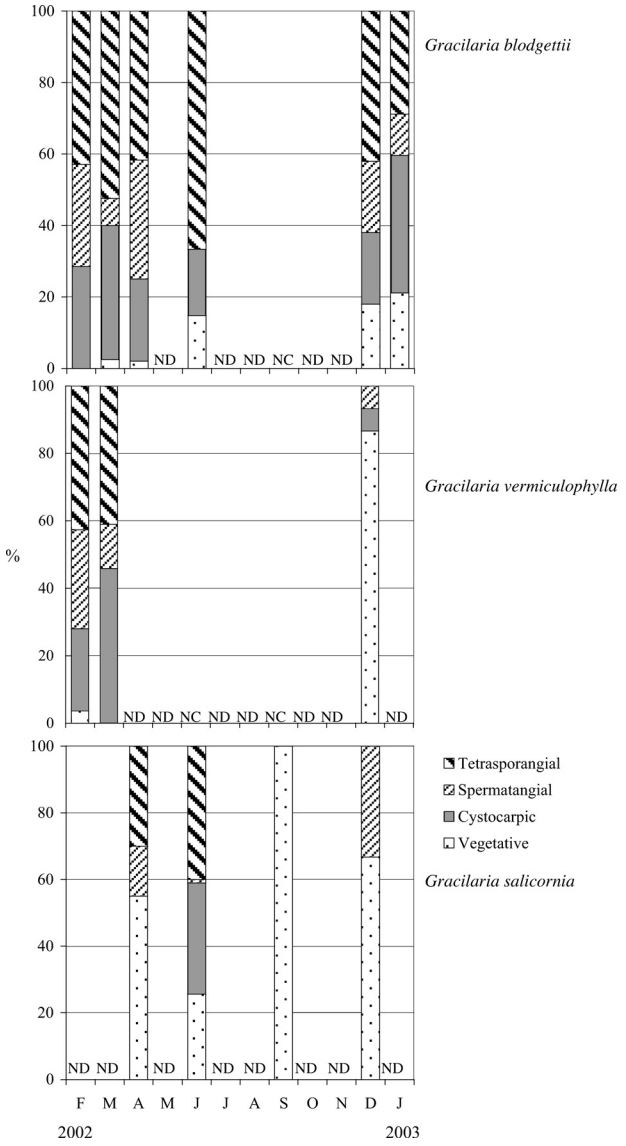


Fig. 5. The ratio of the reproductive and vegetative individuals for three *Gracilaria* species, *G. blodgettii*, *G. vermiculophylla*, and *G. salicornia* from Okinawa Island, southern Japan collected during February 2002 to January 2003. NC indicates [not collected], and ND indicates [No Data], respectively.

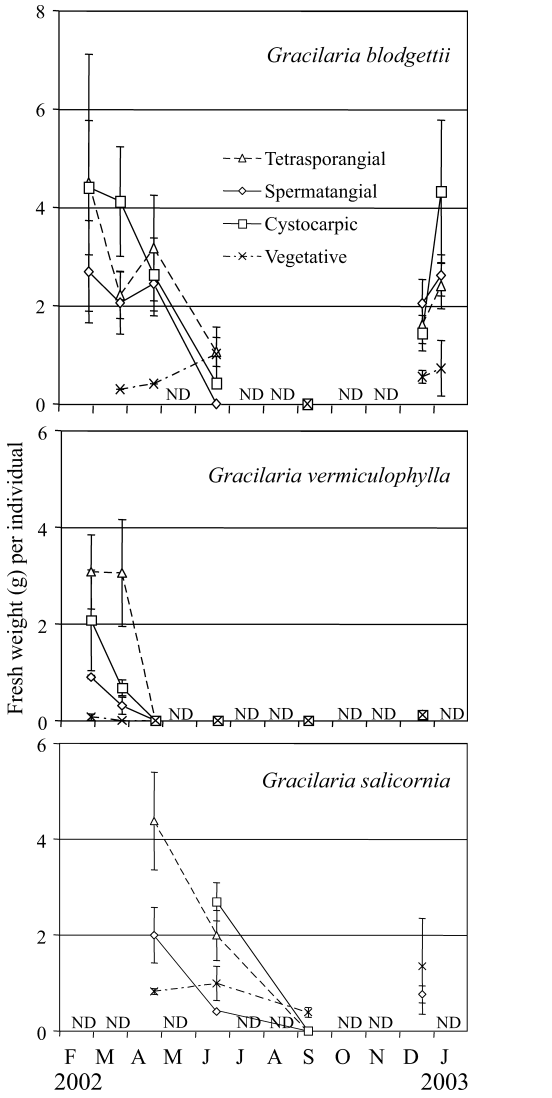


Fig. 6. Seasonal changes in average fresh weight (g) per individual for three *Gracilaria* species, *G. blodgettii*, *G. vermiculophylla*, and *G. salicornia* at Okinawa Island, southern Japan collected during February 2002 to January 2003. Values are given as the mean±S.E.

vegetative plants were present throughout the year, juvenile plants were confirmed only in December at the proportion of 66.7%. The ratio of tetrasporophytes to gametophytes was varied by the collection date. Tetrasporophytes occurred in April and June at 30% and 40%, respectively, and spermatangial plants occurred in December (33%) and March (15%) (Fig. 7). However, cystocarpic plants appeared only in June at 33%. The ratios of total vegetative plants, tetrasporophytes, spermatangial and cystocarpic plants throughout the year were: 46:7:19:28.

Fresh weight during each reproductive phase showed an obvious seasonal peak in April (4.37±1.02 g for tetrasporophytes, and 1.99±0.58 g for spermatangial plants) and June (2.69±0.40 g for cystocarpic plants), then decreased and dis-

Fig. 7. Vertical section of spermatangial conceptacle of *Gracilaria salicornia* collected from Namisato Beach, Kin Town, Okinawa.

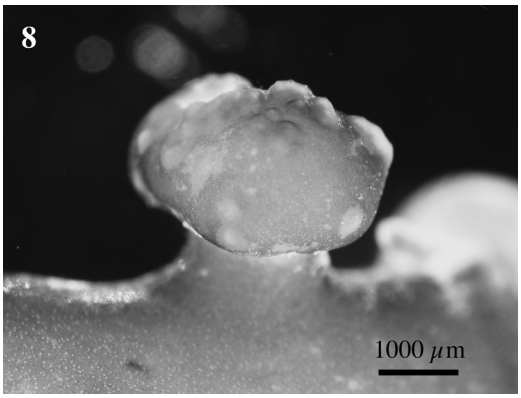


Fig. 8. *Congracilaria babae* Yamamoto from Namisato Beach, Kin, Okinawa Island.

appeared by September (Fig. 6). An adelphoparasitic alga that grows on *G. salicornia*, *Congracilaria babae* Yamamoto (Yamamoto 1986), was confirmed only during June at a rate of 65.6% of the host plants (Fig. 8).

Discussion

Okinawa Is. is located in a subtropical climate and its coastal waters are strongly influenced by the Kuroshio Current that flows along the western edge of the Ryukyu Iss. Three species of *Gracilaria* in this study were generally abundant in winter to spring, and decreased in abundance or were absent during summer, suggesting that temperature and other environmental factors during winter and spring might provide the best habitat for growth and reproduction.

G. blodgettii appeared in December, and decreased in June. The ratio between tetrasporophytes to gametophytes was almost even except for the end of maturation period, and supports an earlier study (Gerung et al. 1997). Although they did not separate distinguish spermatangial plants from vegetative plants because it is difficult to identify spermatangial conceptacles, our result reveals that the ratio between spermatangial and cystocarpic plants was also similar in proportion except near the end of the maturation period.

G. blodgettii is common in the western Pacific Ocean as well as the Caribbean Sea including the type locality, Key West, Florida (Harvey 1853, Fredericq, and Norris 1992, Silva et al. 1996, Terada et al. 2000). Regarding the Southeast and East Asian populations of this species, the Ryukyu Iss. are known as their northern distributional limit and it has never been found in Kyushu Island., which is in the southern region of the Japan mainland. In Kyushu Is., seawater temperatures are about 16°C in winter, our result suggests that this taxon occurred and matured at temperatures between 19°C and 25°C.

Mature plants of *G. vermiculophylla* were confirmed only in December, February and March, and the temperature during this period ranged from 19°C to 22°C (winter season). In this study, the seasonality of this Okinawan plant was different from that of Hokkaido Is., northern Japan, especially in the timing of maturation (Terada et al. 2000). Maturation periods of the plants from Hakodate (41°46′N, 140°45′E), Hokkaido Is., was reported to be in early summer season (June and July). However, the temperature of the maturation period in Hakodate was between 19°C and 24°C, suggesting that the temperature of the maturation period in Okinawa Plant was almost identical with that of Hakodate. They also reported that the matured plant appears in March in southern part of Japan (cf. Kagoshima (31°35′N, 130°36′E) and Awajishima Island (34°16′N, 135°57′E)), and that the water temperature during this month is similar to that of June in Hokkaido.

In this study, *G. vermiculophylla* was found only in the winter season although the other two tropical species were observed until early summer. Given that Okinawa Is. is known as the southern distributional limit for the former (Terada and Yamamoto 2002), we hypothesize that temperature and other environmental factors might restricting the occurrence period of this southern limit population.

G. salicornia was observed between December and September. Although tetrasporophytes mostly dominated in June at rate of 40%, the spermatangial and cystocarpic plants occurred at rates less than 33%, each. We suggest that these differences in ratio were caused by the difference in the timing of the maturation period for each reproductive plant. Note that spermatangial plants might mature earlier than cystocarpic plants. We suggest that spermatangial plants might quickly disappear after the release of their spermatium.

Furthermore, this species is widely distributed in the western Pacific and Indian Ocean (Silva et al. 1996), and is known as an invasive species that has occupied coral substrata throughout the year in Oahu Is., Hawaii (Abbott 1999, Gurgel et al. 2006). The Hawaiian taxon is reported to be usually sterile but at times tetrasporangial and more rarely gametangial organs are observed. Our result indicates that the Japanese taxon has a typical maturation period and disappears during the summer season, suggesting that the growth characteristics and temperature tolerance of Japanese taxon might be different from those of Hawaii. Although spermatangial plant had not been reported regarding Japanese taxon (Ohmi 1958, Yamamoto 1978, Yoshida 1998), we confirmed it for the first time (Fig. 7).

This study revealed that both tropical- and temperate-species of *Gracilaria* occurrs and dominates during the winter and spring season in Okinawa Is. Although we suppose that these characteristics of growth and maturation are mainly influenced by water temperature, further studies are required regarding their temperature tolerance by promoting both photosynthetic and culture experiments.

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