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Original Article

Stocks Discrimination in Lady Fish, *Elops machnata* (Forskal, 1775) from Southeast and Southwest Coast of India Based on Morphometric and Meristic Analysis

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Abstract

Morphometric and meristic analysis of ladyfish, *Elops machnata*, were used to discriminate stocks along the Southeast and Southwest coast estuaries of India. Morphometric and meristic analyses showed a similar pattern of differentiation between *E. machnata* stocks and revealed a clear discreteness of two groups, an East coast (Marakanam, Parangipettai and Muthupettai) population and a West coast (Cochin) population. Higher total length (TL) (28.00 ± 7.043 cm), fork length (FL) (89.27 ± 2.201% TL) and standard length (SL) (81.77 ± 2.582% TL) were recorded in Cochin population and they were significantly different from the other three populations. Meristic counts were relatively homogenous in all the studied populations. No significant variation was found in counts of dorsal fin ray (DFR), anal fin ray (AFR), pectoral fin ray (PFR) and pelvic fin ray (PLFR). The first and second components (PCA analysis) accounted for about 92.2% of variation in all the morphometric characters. Among them, pre pectoral length (PPL) and pre dorsal length (PDL) showed high loading values in PC1 in all four populations. The overall random assignment of individuals to their original group was higher in morphometric than in meristic analysis. Such a presumption could be authenticated henceforth with molecular markers. Hence, further studies, using molecular markers are still required to precisely evaluate the genetic structure of *E. machnata* along the Indian coast.

Keywords: *Elops machnata*; meristic; ladyfish; morphometric; population structure

Introduction

Elops machnata belongs to the family Elopidae, which forms part of the order Elopiformes. The species is widely distributed in tropical-subtropical, marine and coastal waters (McBride *et al*., 2010). In terms of conservation status, *E. machnata* is listed as a species of least concern (LC) in the International Union for Conservation of Nature (IUCN) red list (Adams *et al*., 2018). *Elops* species are important components of global fisheries, in either commercial, recreational or subsistence sectors (McBride *et al*., 2010).

Morphometric analysis has been applied to many stock differentiation and life-history problems in many fish species (Bronte *et al*., 1999). If there is any morphological variation in various populations of a particular species, one can discriminate the morphotypes, and such variation may be useful in assessing the stock structure of populations (Joseph and Jayasankar, 2001). Those morphological differences within a morphotype may indicate geographically isolated stocks, whose shapes may be influenced based on local environmental conditions or by genetic bases (Joseph and Jayasankar, 2001).

Different populations of the same fish species are often different in phenotypic characters (Pakkasmaa and Piironen, 2001). Morphological differentiations can principally result from two causes; genetic differences or environmental factors, or their interactions. Genetic differences and reproductive isolation between populations can lead to local adaptation, which is reflected in morphology, behaviour, physiology and life history traits (Taylor, 1991; Pakkasmaa and Piironen, 2001; Kara *et al*., 2011). Environmental factors, on the other hand, can produce phenotypic plasticity, which is the capacity of a genotype to produce different phenotypes in different environmental conditions (Scheiner, 1993). Morphometric assessment is not only essential to understand the taxonomy

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but also the fitness of a species (including reproduction) in a habitat. The shape and structures are unique to a particular species and the variations in morphological characters are probably related to the habit and habitat conditional variants of this species (Cavalcanti *et al*., 1999).

In this study, morphometric and meristic characters were analyzed in order to investigate possible variation in four populations of *E. machnata* in Southeast and Southwest coast of India.

Materials and Methods

Animal collection and site description

Four populations including Marakanam (12° 12′ 0″ N, 79° 57′ 0″ E), Parangipettai (11° 30′ 33″ N, 79° 43′ 13″ E) and Muthupettai (10° 23′ 0″ N, 79° 30′ 19″ E) estuaries of Southeast coast and Cochin (9° 58′ 4.8″ N, 76° 14′ 38.4″ E) estuary of Southwest coast of India were selected for the hereby study (Fig. 1).

In total, 390 individuals were collected from four populations *viz*., Marakanam (105), Parangipettai (100), Muthupettai (110) and Cochin (75). Samples were collected randomly from the respective estuary landing centres. All fishes were identified using the FAO Fish Identification Sheets (Thomson, 1984) and further confirmation was carried out at Zoological Survey of India, Southern Regional Centre, Chennai.

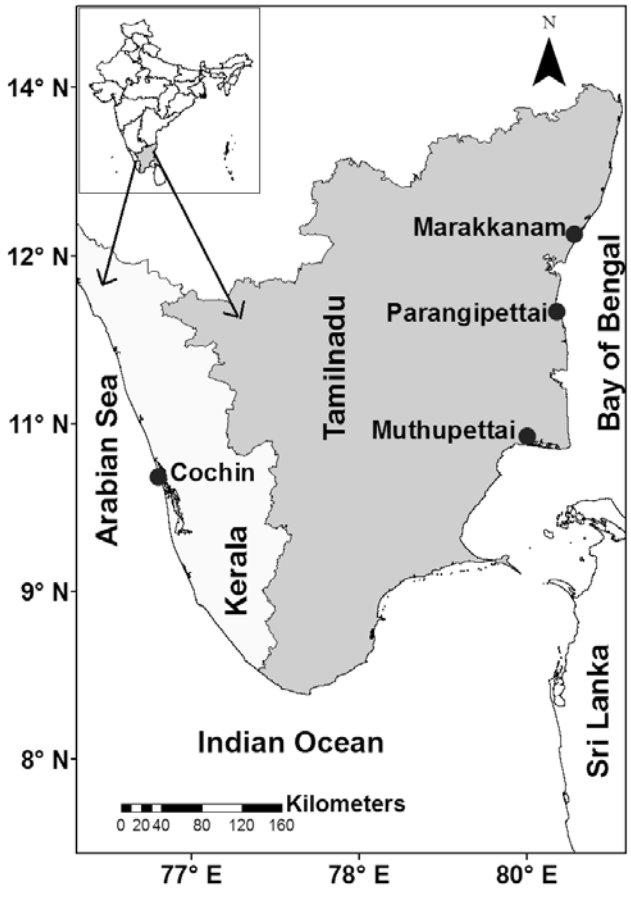

Fig. 1. Map showing the collection sites of *E. machnata*

Morphometric measurement

As per standard protocol (Motomura *et al*., 2001), 25 morphometric characters and four meristic counts were selected for phenotypic analysis. Morphometric measurements were taken using digital calliper (Mitutoyo, Japan) with 0.1 mm accuracy. These characters included: TL: Total length; FL: Fork length; SL: Standard length; PDL: Pre-dorsal length; PPL: Pre-pectoral length; PPLL: Pre-pelvic length; PAL: Pre-anal length; HL: Head length; HH: Head height; UJL: Upper jaw length; EH: Eye height; OD: Orbit diameter; MBH: Maximum body height; MBW: Maximum body width; BDFL: Base of dorsal fin length; BAFL: Base of anal fin length; BPFL: Base of pectoral fin length; BPLFL: Base of pelvic fin length; ODFLAFL: Origin of dorsal fin length to anal fin length; OPLFLAFL: Origin of pelvic fin length to anal fin length; CUPL: Caudal peduncle length; CUPH: Caudal peduncle height; CUPW: Caudal peduncle width; UCFL: Upper caudal fin length; LCFL: Lower caudal fin length; DFR: Dorsal fin ray; AFR: Anal fin ray; PFR: Pectoral fin ray; PLFR: Pelvic fin ray. Measurements were taken from the left side of individual fish and efforts were made to maximize consistency (Fig. 2). All the length measurements were taken between identified points along the anterior-posterior axis, whereas depth measurements of the body were taken perpendicularly between the identified points. Each meristic count was taken twice on the same specimen using a hand-held magnifying lens.

All the morphometric data of the four populations were analyzed using univariate analysis of variance (ANOVA), with Tukey HSD (for unequal N) post-hoc comparison tests to investigate the significant morphometric difference. Tests were considered significant at $P<0.05$ level. Multivariate statistical analysis method of principal component analysis (PCA) was performed in statistical package PAST (version 2.14) to discriminate the populations based on grouping of components significantly using all morphometric measurements.

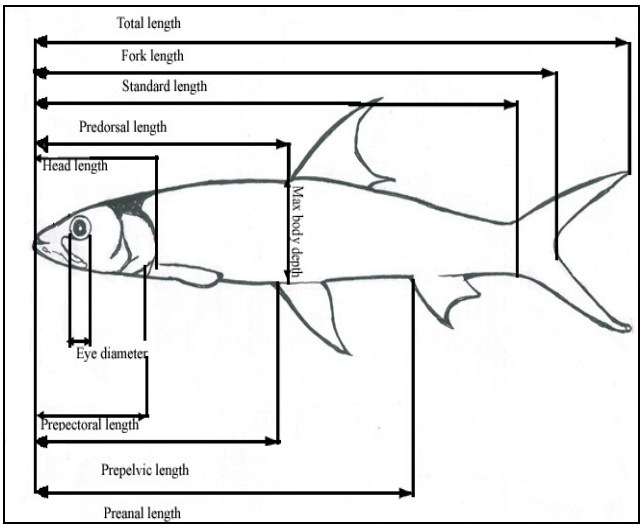

Fig. 2. Schematic diagram of fish showing superficial landmark points used in measuring morphometric characters within the study

Results and Discussion

Morphometric and meristic characters

The details of the morphometric characters of *E. machnata* from four populations are shown in Table 1. There were significant differences in some morphometric characters of the populations studied. Higher TL (28.00 ± 7.043 cm), FL (89.27 ± 2.201% TL) and SL (81.77 ± 2.582% TL) were recorded in Cochin population and it was significantly different from the other three populations. The MBW in Cochin population (18.31 ± 5.746 mm) significantly deviated from the other three Tamilnadu populations. Higher value of HH (66.83 ± 2.690% MBH), MBH (40.54 ± 7.445 mm), BPLFL (3.48 ± 0.398% TL) and ODFLAFL (24.30 ± 1.302% TL) were observed in Parangipettai population. It was significantly different from the other three populations. The values of OD (41.99 ± 2.672% HH) and OPLFLAFL (20.73 ± 1.362% TL) were high in Muthupettai population and significantly deviated from other three populations. In Marakanam population the PDL, PPLL, CUPL and UCFL showed prominent values compared with the other three populations. The *F* value was more for UJL (165.224 % HL) and EH (371.687 % HH) among the four populations. Meristic counts were relatively homogenous in all the studied populations. No significant variation was found in counts of DFR, AFR, PFR and PLFR (Table 2).

Table 1. Morphometric characters in four populations of *E. machnata*

Variable	Mean ± Std. Deviation (Std. Error)				*F* value
	Parangipettai	Muthupettai	Marakanam	Cochin	
TL (cm)	26.73 ± 4.748^{a} (0.475)	23.67 ± 3.965^{a} (0.396)	20.81 ± 3.416^{a} (0.342)	28.00 ± 7.043^{b} (1.381)	37.979
FL (%TL)	84.18 ± 1.351^{a} (0.135)	84.31 ± 1.346^{a} (0.135)	85.56 ±1.424^{a} (0.142)	89.27 ± 2.201^{b} (0.432)	97.481
SL (%TL)	78.74 ± 2.121^{a} (0.212)	77.99 ± 1.275^{a} (0.128)	78.50 ± 1.801^{a} (0.180)	81.77 ± 2.582^{b} (0.506)	29.372
PDL (%TL)	41.85 ± 1.336^{a} (0.134)	41.73 ± 1.254^{a} (0.125)	43.14 ± 1.450^{b} (0.145)	40.77 ± 1.505^{a} (0.295)	31.273
PPL (%TL)	18.52 ± 1.096^{c} (0.110)	18.36 ± 1.000^{a} (0.100)	19.37 ± 1.195^{b} (0.119)	18.35 ± 1.263^{a} (0.248)	16.897
PPLL (%TL)	41.30 ± 1.347^{a} (0.135)	40.98 ± 1.216^{a} (0.122)	42.29 ± 1.201^{b} (0.120)	40.72 ± 1.449^{a} (0.284)	22.274
PAL (%TL)	61.08 ± 1.698^{a} (0.170)	60.26 ± 0.991^{a} (0.099)	61.52 ± 1.720^{b} (0.172)	60.58 ± 1.793^{a} (0.352)	12.094
HL (%TL)	19.40 ± 1.092^{c} (0.109)	19.01 ± 1.425^{a} (0.142)	19.37 ± 0.981^{a} (0.098)	19.69 ± 1.158^{b} (0.227)	3.329
HH (%MBH)	66.83 ± 2.690^{b} (0.269)	64.30 ± 2.389^{a} (0.239)	65.79 ± 1.996^{a} (0.200)	65.23 ± 3.374^{a} (0.662)	17.928
UJL (%HL)	45.21 ± 2.783^{a} (0.278)	46.94 ± 1.819^{a} (0.182)	50.87 ± 2.722^{a} (0.272)	55.58 ± 3.075^{a} (0.603)	165.224
EH (%HH)	54.75 ± 2.768^{a} (0.277)	56.42 ± 2.727^{a} (0.273)	60.80 ± 2.578^{a} (0.258)	41.81 ± 1.721^{a} (0.337)	371.687
OD (%HH)	39.05 ± 2.037^{a} (0.204)	41.99 ± 2.672^{b} (0.267)	40.13 ± 2.773^{a} (0.277)	41.81 ± 2.654^{a} (0.520)	25.613
MBH (mm)	40.54 ± 7.445^{b} (0.744)	34.99 ± 7.199^{a} (0.720)	30.24 ± 6.706^{a} (0.671)	39.88 ± 12.947^{a} (2.539)	32.475
MBW (mm)	13.22 ± 3.636^{a} (0.364)	11.09 ± 1.975^{a} (0.198)	10.45 ± 2.162^{a} (0.216)	18.31 ± 5.746^{b} (1.127)	54.013
BDFL (%TL)	12.53 ± 0.750^{c} (0.075)	11.87 ± 0.818^{a} (0.082)	12.41 ± 1.031^{b} (0.103)	11.96 ± 1.147^{a} (0.225)	11.270
BAFL (%TL)	7.58 ± 0.635^{c} (0.063)	7.17 ± 0.556^{a} (0.056)	7.33 ± 0.651^{a} (0.065)	7.77 ± 0.633^{b} (0.124)	11.011
BPFL (%TL)	3.51 ± 0.379^{c} (0.038)	3.09 ± 0.424^{a} (0.042)	3.22 ± 0.343^{a} (0.034)	3.42 ± 0.574^{b} (0.113)	20.223
BPLFL (%TL)	3.48 ± 0.398^{b} (0.040)	3.14 ± 0.401^{a} (0.040)	3.26 ± 0.363^{a} (0.036)	3.37 ± 0.520^{a} (0.102)	12.715
ODFLAFL (%TL)	24.30 ± 1.302^{b} (0.130)	23.64 ± 1.178^{a} (0.118)	24.27 ± 1.349^{a} (0.135)	19.03 ± 1.123^{a} (0.220)	131.849
OPLFLAFL (%TL)	20.35 ± 1.613^{c} (0.161)	20.73 ± 1.362^{b} (0.136)	20.14 ± 1.808^{a} (0.181)	19.14 ± 1.216^{a} (0.238)	7.539
CUPL (%TL)	5.10 ± 0.834^{a} (0.083)	5.07 ± 0.460^{a} (0.046)	5.55 ± 0.815^{b} (0.082)	4.04 ± 0.458^{a} (0.090)	32.296
CUPH (%MBH)	44.52 ± 4.420^{a} (0.442)	48.00 ± 1.828^{b} (0.183)	47.16 ± 2.194^{a} (0.219)	47.58 ± 2.254^{c} (0.442)	25.584
CUPW (%MBW)	43.98 ± 4.738^{a} (0.474)	43.69 ± 1.935^{a} (0.193)	51.28 ± 2.454^{b} (0.245)	44.37 ± 1.696^{c} (0.333)	124.870
UCFL (%TL)	22.89 ± 0.975^{a} (0.097)	22.29 ± 1.322^{a} (0.132)	23.40 ± 1.557^{b} (0.156)	22.56 ± 1.711^{a} (0.335)	11.739
LCFL (%TL)	21.68 ± 0.918^{c} (0.092)	21.35 ± 0.817^{a} (0.082)	22.16 ± 1.204^{b} (0.120)	21.65 ± 1.809^{a} (0.355)	9.476

Values along the rows not sharing common superscript are significantly different at $P< 0.05$.

Table 2. Meristic characters in four populations of *E. machnata*

Location	DFR	AFR	PFR	PLFR
Marakanam	22-25	14-17	14-18	13-16
Parangipettai	24-25	14-16	16-18	14-16
Muthupettai	23-26	14-16	16-17	13-15
Cochin	22-24	16	16	17

Principal component analysis (PCA)

In PCA analysis, Principal Component 1 (PC1) and Principal Component 2 (PC2) were obtained from all morphological characters and the four populations were partially clustered (Fig. 3). The principal component analysis score plot exhibited that the East coast populations (Marakanam, Parangipettai and Muthupettai) were clustered tightly together. Whereas, the Westcoast population (Cochin) lied separately in the score plot. The first and second components explained about 92.2% and 1.1% variation respectively. Characters such as EH, PPL and PDL showed high loading values in PC1 among the four populations. Within population, individuals were more tightly clustered than the individuals between the populations. This indicates that morphological characters were highly similar in individuals within populations compared with individuals between the populations. Partial homogeneity observed in Parangipettai, Muthupettai and Marakanam clusters and Cochin and Marakanam may be attributed to lack of significant difference in some morphological characters among these populations.

Table 3. Variations associated with principal components and sum of squared loadings for the morphometric measurements of four populations of *E. machnata*

Variables	Component	
	PC1	PC2
TL	-0.846	0.222
FL	-0.101	0.293
SL	-0.257	0.359
PDL	0.579	0.371
PPL	0.604	0.261
PPLL	0.533	0.453
PAL	0.305	0.534
HL	0.221	0.371
HH	0.053	0.208
UJL	0.059	-0.002
EH	0.731	-0.023
OD	0.000	-0.425
MBH	-0.779	0.315
MBW	-0.770	0.299
BDFL	0.038	0.552
BAFL	-0.212	0.494
BPFL	-0.230	0.696
BPLFL	-0.190	0.693
ODFLAFL	0.364	0.244
OPLFLAL	0.083	0.047
CUPL	0.510	0.046
CUPH	0.139	-0.210
CUPW	0.524	0.200
UCFL	0.193	0.327
LCFL	0.164	0.328
Extraction Sums of Squared Loadings		
% of Variance	18.209	13.551
Cumulative %	18.209	31.760
Total	4.552	3.388

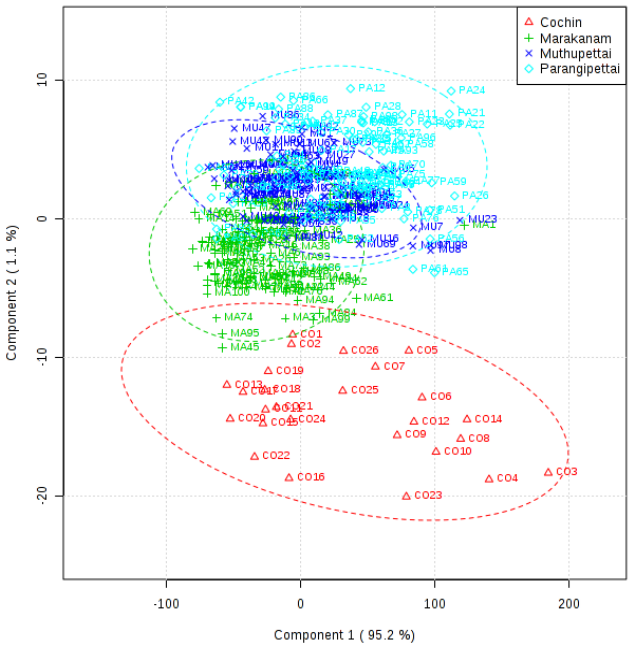

Fig. 3. Plot for first and second principal components in four populations of *E. machnata*

The loading factors for component 1 and component 2 for all morphological characters are shown in Table 3. The PC1 showed 18.209% of variance and PC2 showed 13.55% variance.

Identification of stock structure through phenotypic measurements has been widely used in several fish populations (Uiblein, 1995; Hurlbut and Clay, 1998). Generally, fishes possess high phenotypic plasticity, which has relatively higher coefficients among the population for variation of phenotypes among the vertebrates (Carvalho, 1993). This phenotypic plasticity of fishes likely to be associated with relationship with the changing environmental factors (Wimberger, 1991; 1992). In the present study, the environmental factors of the studied area were not included. However, it is well known that those estuaries are continuously facing different stress conditions due to rapid industrialization.

The East coast populations are close enough when compared to West coast population and exhibiting low phenotypic differentiation in PCA scatter plot analysis. The obtained *P*-values of morphometric results were insignificant to support the established differentiation between these four populations that often leads to taxonomic uncertainty. At the same time, few of the Marakanam individuals clustered with Cochin population and more individuals clustered in a separate place in the plot. Many individuals of Parangipettai and Marakanam population were placed in between the Muthupettai populations and the remaining individuals dropped in a separate cluster in the plot. The close distribution of these samples may be accounted for recent separation due to ecological alterations.

Turan *et al*. (2006) studied the morphological variation of *Pomatomus saltatrix* based on morphometric and meristic analysis of samples collected in Black Seas, Marmara, Aegean and Eastern Mediterranean Seas and the results indicated the existence of three morphologically differentiated groups. Erguden *et al*. (2009) undertook morphometric and meristic analysis of chub mackerel, *Scomber japonicus* in the same locations and they observed a clear pattern of morphometric and meristic differentiation between the stocks. The present study also showed a tentative pattern of differentiation between the stocks and revealed two groups, the East coast estuary stock (Parangipettai, Marakanam and Muthupettai) and the West coast estuary stock (Cochin).

The study observed a low heterogeneity in some morphometric characters of *E. machnata* populations (Table 1). Similarly, the significant spatial heterogeneity was found in some estuarine populations of *Arius jella* in Sri Lanka (Gunawickrama, 2007) and *Etroplus maculates* populations (Manimegalai *et al*., 2010) in India. Generally the morphological variation is expected to be under the isolation by distance model. Within the present study though, the population variations did not agree with that model; such a result might be raised by environmentally induced phenotypic variations due to the different gene expression pattern like in killifish populations (Schulte, 2001). The difference in the gene expression pattern may drive the non-selective plasticity in traits rather than genetic differences among populations, resulting in phenotypic variations (Ayrinhac *et al*., 2004; Schoville *et al*., 2012).

There were no significant differences observed in meristic characters such as rays of dorsal, pectoral, pelvic and anal fins of the four populations in the present investigation. These results suggest that environmental variations have no influence on these meristic characters. Similarly, there was no variation found in the meristic characters of the populations of *Clupea harengus* sampled from different environmental conditions in Baltic Sea (Jorgensen *et al.,* 2008) and *Arius jella* collected from different estuaries of Sri Lanka (Gunawickrama, 2007). These results suggest that variations arising in meristic characters may take long periods of evolution.

The present study found that no large phenotypic differences among the population indicate that any restriction on gene flow that may occur on these population units is not sufficient to maintain them in complete isolation. However, some morphological characters of the present study showed significant heterogeneity among populations. These observed variations may have occurred due to partial isolation of populations or local adaptations changed by differential gene expression pattern (Schoville *et al*., 2012). Moreover, the present study recommends that population differentiation through morphological characters could not be considered since homogeneity exists in the four *E. machnata* populations. Morphological divergence has been reported in the estuarine fish population that are not completely geographically separated, suggesting that partial isolation may play a role in population sub-divisions (Manimegalai *et al*., 2010). Their study can also explain that fishes living in the same place showed morphological divergence. It would indicate the possibility for micro-habitat restriction that may influence this variation. However, it is now commonly accepted that morphological variation has both environmental and genetic components. Thus, morphometric differences may reflect genetic differences between the stocks and/or environmental differences between localities. Therefore, stock identification based on morphological characters must be confirmed by genetic evidence to verify that the phenotypic differences reflect some degree of reproductive isolation rather than simply environmental differences. The present analysis does not include environmental data for the sample localities, thus it is not possible to confirm whether the observed variation is associated with environmental conditions, and therefore, further environmental comparisons of these areas would be worthwhile.

Conclusions

In conclusion, based largely on morphological differences, there are two groups of ladyfish in the estuary waters of India, a West coast group and an East coast group. Although environmental factors may govern to some degree the potential morphological differentiation of lady fish aggregations, the detected pattern of differences at least shows that there is some restriction to intermingling between stocks (no data, only assumption). Further understanding of differentiation must include broader samplings throughout the species range, collections of molecular genetic data such as RAPD, microsatellites and physical tagging programs may be designed to measure long-distance dispersal of *E. machnata*.

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