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Host specificity and multivariate diagnostics of cryptic species in predacious cheyletid mites of the genus *Cheletophyes* (Acari: Cheyletidae) associated with large carpenter bees

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Three closely related species of mites of the genus *Cheletophyes* were collected from large African carpenter bees: *C. venator* (Vitzthum) is associated with *Xylocopa nigrita*; ***C. torridae* sp. nov.** and ***C. mbomba* sp. nov.** are associated with *Xylocopa torrida*. Ranges of 21 morphometric variables in *C. venator* and putative *C. torridae* are overlapping. However, a large gap between the two groups was detected in multivariate shape space, indicating the presence of two sibling species. The best subset canonical variates analysis (CVA) produced a model with fewer predictors and higher classification accuracy compared to other tested approaches, stepwise CVA and elimination of variables based on absolute correlation with canonical function. The model differentiates the two sibling species with 100% accuracy in jackknife resampling and external validation ($N = 100$) using four variables. A logistic regression model built as an alternative to CVA has two variables and 97.6% and 100% classification accuracy for the analysis sample and external validation, respectively. Both models are available online at http://insects.ummz.lsa.umich.edu/beemites/morphometrics.html. Host specificity of predacious *Cheletophyes* to their bee hosts in the community that includes bees, cleptoparasitic mites and their predators is discussed. Formal descriptions and synonymy of the species based on the results of the analyses are also given. *Biological Journal of the Linnean Society*, 2006, **87**, 45–58.

ADDITIONAL KEYWORDS: acarinarium – Apidae – canonical variates analysis – logistic regression analysis – *Xylocopa nigrita* – *Xylocopa torrida*.

INTRODUCTION

Although some cases of host specificity among mutualistic scavengers, parasitic or cleptoparasitic mites and their bee hosts are well-known (Eickwort, 1994), it may be that the evolutionary ties of many predatory mites and bees are much weaker in these cases because the mites do not directly depend on resources provided by the bee other than the physical habitat of the nest cells. On the other hand, developing mutualistic associations with predatory mites might provide some selective advantages to such bees because the mites may reduce the number of cleptoparasites or parasites and thus, potentially increase fitness. The host specificity of nidicolous predatory mites in such situations has never been tested, and the matter is complicated by the fact that many mites associated with closely related hosts may represent cryptic species. In this paper, we explore both problems with mites of the genus *Cheletophyes*.

Mites of the genus *Cheletophyes* (Acari: Cheyletidae) are obligatory associates of carpenter bees of the genus *Xylocopa* (Apidae: Xylocopinae); the few records from other hosts are probably accidental. Seven species are distributed in the Old World, and one species, *C. panamenis* Klompen, Méndez et Lukoschus, was described from Panama (Fain & Bochkov,

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2001a). All stages of these mites are nidicolous predators, feeding on different microarthropods, including some cleptoparasitic astigmatid mites (Shereef & El-Duweini, 1980; Eickwort, 1994). Females of Old World species of *Cheletophyes* disperse in mesosomal acarinaria of female bees. OConnor (1993) hypothesized that the relationships between *Cheletophyes* and their bee hosts are mutualistic, and the bees have developed mesosomal acarinaria to transfer the predacious mites controlling nest cleptoparasites.

In the present study we examine species of *Cheletophyes* associated with two large carpenter bees, *Xylocopa nigrita* (Fabricius) and *X. torrida* (Westwood) widely distributed in sub-Saharan Africa. Three closely related mite species belonging to a newly recognized lineage (species complex *venator*) were found: *C. venator* (Vitzthum) is associated with *X. nigrita* and two new morphotypes are associated with *X. torrida*. The status of one of the new morphotypes was initially uncertain because ranges of its morphometric characters overlap those of *C. venator*.

We employed methods of multivariate morphometrics to determine whether these differences are influenced by strong host effects or if there are two sibling species that possibly coevolved with two different bee species. The presence of broadly overlapping geographical ranges in the host distributions allowed us to rule out possible geographical effects on the model. Formal descriptions of the species based on the results of canonical variates and logistic regression analyses are also given.

MATERIAL AND METHODS

Collections

A total of 336 mite specimens were collected from 22 dried museum specimens of *X. nigrita* and *X. torrida* from Ivory Coast, Cameroon, Democratic Republic of Congo, Tanzania, and South Africa, one freshly collected specimen of *X. nigrita* from Tanzania, and three bulk samples from multiple speciemsns of *X. torrida* from Congo (Fig. 1; see material under species description below). Intensive sampling of sympatric populations of the two bee species was conducted from two localities in Congo and Cameroon. Each specimen was labelled 'Mites removed' and numbered, uniquely identifying the bee hosts. Depositories of these specimens are indicated under the species descriptions below.

Mite specimens, including types, are deposited in the following institutions: Institut royal des Sciences naturelles, Brussels, Belgium (IRSNB), Musée royal de l'Afrique Centrale, Tervuren, Belgium (MRAC), University of Michigan Museum of Zoology, Ann Arbor, MI (UMMZ), University of Kansas Natural History Museum (KU), Zoological Institute, Russian Academy of Science, St. Petersburg, Russia (ZIN), and the Hungarian Natural History Museum, Budapest, Hungary (HNHM).

Morphometric analyses

Mite specimens were cleared in Nesbitt's fluid and mounted dorsoventrally in Hoyer's medium using standard methodology (OConnor & Houck, 1991). Continuous morphological characters were measured with an ocular micrometer using a Zeiss microscope with phase contrast optics and converted to micrometers prior to statistical analyses. Twenty-three measurements were made (Table 1). We did not measure legs and leg setae because these structures can be easily damaged during slide preparation.

On the basis of the ranges of variation in several characters, the specimens were allocated to three putative morphotypes. The first, *Cheletophyes mbomba* sp. nov., exhibited distinct differences in several qualitative and quantitative characters (see description below). Two others were distinguishable only by their group means; they occurred on different bee species, indicating either strong host-related variance in the single mite species, *C. venator*, or the presence of two sibling species. To test these hypotheses we employed methods of multivariate morphometrics.

Measurements of 125 individuals of the two putative groups were used for the initial investigation of the discriminatory properties of the variables relative to the efficacy of data collection. The latter was estimated by a number of missing values. Two variables with more than 10.1% and 11.1% of missing values for groups 1–2, respectively, were dropped from the model. For the remaining 21 variables (Table 1), missing values were replaced by values predicted by a linear regression, where a variable with missing data was considered the response, and the variable 'length of idiosoma' was considered as the predictor.

Values in the resulting matrix were converted to Darroch and Mosimann shape variables (Darroch & Mosimann, 1985) to suppress the overall size factor and create scale-free, or dimensionless, variables. A critical evaluation of this and other methods of size adjustment was made by Jungers *et al.* (1995). In contrast to the shape component, the size component is influenced mostly by nongenetic variance and not useful in discrimination of species. In the present analysis, we follow Darroch & Mosimann (1985) and explicitly define size as the geometric mean of all variables.

Morphometric analyses were done with SPSS v.10.0.7a for Macintosh (SPSS Inc., Chicago IL).

Principal component analyses

PCAs were conducted on variance-covariance matrices of log raw data and log shape variables to determine

Table 1. Measurements of 23 morphological structures of three *Cheletophyes* species. *Variables used in the 21-variable analyses. **Variables used in the 4-variable analyses

Variable		venator	torridae	mbomba
*	idiosoma, length	263–380 (332.1 ± 19.97, $N = 80$)	328–410 (352.3 ±19.03, $N = 45$)	298–357 (327.6 ± 15.84, $N = 10$)
*	idiosoma, width	205–287 (245.2 ± 20.49, $N = 78$)	216–351 (257.8 ±25.50, $N = 40$)	211–246 (228.2 ± 11.03, $N = 10$)
**	propodosomal shield, length	135–211 (176.6 ± 11.12, $N = 153$)	164–216 (185.7 ±11.32, $N = 72$)	140–176 (154.4 ± 11.10, $N = 10$)
*	gnathosomal length	140–176 (161.3 ± 8.82, $N = 80$)	146–187 (166.5 ±8.87, $N = 45$)	140–152 (145.7 ± 3.32, $N = 10$)
	vi	46–70 (59.2 ± 5.76, $N = 68$)	66–88 (74.9 ± 5.95, $N = 41$)	51–64 (54.8 ± 4.46, $N = 9$)
*	*ve*	46–73 (60.4 ± 5.73, $N = 73$)	66–90 (76.9 ± 6.35, $N = 41$)	51–64 (56.2 ± 3.83, $N = 9$)
*	*sci*	44–75 (60.5 ± 6.37, $N = 75$)	66–88 (78.1 ± 5.34, $N = 40$)	53–57 (54.5 ± 1.47, $N = 9$)
*	*sce*	46–77 (62.0 ± 6.63, $N = 79$)	66–92 (80.6 ± 6.37, $N = 41$)	53–55 (54.3 ± 1.10, $N = 9$)
*	*h*	57–88 (74.7 ± 6.43, $N = 76$)	77–110 (95.4 ± 7.19, $N = 43$)	66–73 (67.5 ± 2.33, $N = 10$)
*	*d1*	42–66 (55.7 ± 6.56, $N = 77$)	59–84 (73.3 ± 5.32, $N = 43$)	44–46 (44.7 ± 1.06, $N = 10$)
*	*N1*	37–62 (51.7 ± 5.90, $N = 76$)	55–81 (69.3 ± 5.98, $N = 43$)	40–46 (43.1 ± 2.58, $N = 10$)
*	*N2*	37–70 (53.6 ± 7.58, $N = 78$)	57–92 (71.4 ± 7.45, $N = 45$)	40–46 (42.2 ± 2.50, $N = 10$)
**	*d2*	37–66 (52.1 ± 6.04, $N = 151$)	55–88 (73.7 ± 6.62, $N = 71$)	37–44 (40.9 ± 1.86, $N = 10$)
*	*d3*	42–62 (52.1 ± 5.31, $N = 77$)	55–86 (67.8 ± 7.25, $N = 44$)	33–46 (38.1 ± 4.02, $N = 10$)
*	*d4*	35–55 (45.0 ± 5.06, $N = 80$)	46–70 (57.4 ± 5.71, $N = 45$)	33–42 (36.5 ± 2.97, $N = 10$)
*	*d5*	35–57 (48.2 ± 5.23, $N = 74$)	46–70 (55.7 ± 5.08, $N = 41$)	37–48 (42.3 ± 3.61, $N = 9$)
*	*l1*	46–77 (61.9 ± 6.46, $N = 78$)	73–95 (81.7 ± 6.16, $N = 43$)	46–59 (52.1 ± 4.02, $N = 10$)
**	*l2*	44–70 (57.2 ± 5.59, $N = 151$)	64–88 (77.1 ± 5.22, $N = 70$)	44–46 (44.9 ± 1.14, $N = 10$)
*	*l3*	42–68 (55.6 ± 5.91, $N = 76$)	62–88 (73.6 ± 5.86, $N = 41$)	44–55 (47.7 ± 3.60, $N = 10$)
**	*l4*	40–62 (52.0 ± 5.08, $N = 145$)	59–77 (67.2 ± 4.44, $N = 69$)	46–55 (50.8 ± 3.80, $N = 10$)
	l5	26–40 (32.1 ± 2.51, $N = 77$)	29–42 (34.3 ± 2.87, $N = 38$)	29–37 (32.1 ± 3.47, $N = 10$)
*	pygidial shield, length	37–66 (50.5 ± 6.58, $N = 79$)	44–70 (58.2 ± 8.45, $N = 45$)	70–92 (78.3 ± 8.24, $N = 10$)
*	pygidial shield, width	33–51 (41.1 ± 4.05, $N = 79$)	33–48 (40.0 ± 2.99, $N = 45$)	66–77 (70.0 ± 4.61, $N = 10$)

the extent to which overall differences among individuals could be attributed to a combination of size and shape vs. shape only (Darroch & Mosimann, 1985; Simmons, Falsetti & Smith, 1991). Because PCA on log raw data resulted in one component only, both analyses were forced to extract four principal components. PC4 and beyond probably represent measurement errors (Houck & OConnor, 1998).

Canonical variates analyses

CVAs were conducted to: (1) select the smallest set of variables that has the highest precision in classification (variable selection), and (2) develop a classification rule for discrimination of the morphotypes. Because previously conducted PCAs showed that size-and-shape variables were as useful for discrimination as shape variables, CVAs were conducted on log measurements. Variables were entered together (simultaneous CVA) using equal prior probabilities.

Variable selection

We used several methods for assessing the contribution of the predictor variables to group discrimination.

1. Discriminant loadings (Hair *et al*., 1998). The variable with the smallest correlation with the canonical function was dropped from the model, and for the remaining variables a new covariance matrix was constructed and subjected to a new CVA; the procedure is iterative.
2. Stepwise backward CVA based on *F*-values.
3. Best-subset method (Huberty, 1994) based on the jackknife misclassification rate.

Validation of results

A canonical variate was derived from the original data using the jackknife method to assess the classification accuracy rate (Huberty, 1994; Lance, Kennedy & Leberg, 2000). Because the sample size was relatively small and the number of predictors comparatively large, we did not divide the cases into analysis (training, calibration) and holdout samples. An additional sample of the two putative morphotypes was employed as the holdout sample to estimate the external validity of the function derived from the reduced subset of variables. The holdout sample included 100 specimens: 73 specimens of *C. venator* representing three mixed samples collected from multiple individuals of *X. nigrita* and 27 specimens of *C. torridae* from Congo.

Logistic regression

This analysis is used in place of CVA as it usually involves fewer violations of assumptions, is robust, and has coefficients that are easier to interpret. Logistic regression is preferred when data are heteroscedastic, not normal in distribution or group sizes are very unequal (Hair *et al*., 1998). Although the analysis overcomes several violated assumptions of CVA, some other assumptions still apply, for example, no multicollinearity and large samples (Menard, 2001). Logistic regression is very common in ecological studies, but there are only a few applications of this technique to morphometric data in zoology. Only five papers on this subject have been published in the past five years and the results were not compared with CVA (DeMartini & Lau, 1999; Groger, 2000; Rodriguero *et al*., 2002; Caceres-Martinez *et al*., 2003; Scheurer, Bestgen & Fausch, 2003).

We conducted both logistic regression and CVA to evaluate and compare their potential advantages and disadvantages for morphometric data. The presence of multicollinearity and small sample size allowed us to analyse only three variables. From the final model developed by CVA (see below), three variables with the highest absolute loadings were selected, *l2*, *d2*, *l4*. One variable, *d2* was also eliminated on the basis of Wald and likelihood ratio statistic ($P = 0.533$ and 0.524, respectively). The analysis and holdout samples are the same as for CVA. Raw data, including the holdout sample, are available at http://insects.ummz.lsa.umich.edu/beemites/Morphometrics/Cheletophyes.htm.

All measurements are in micrometers (μm). Statistical data are presented as range, mean ± standard deviation.

RESULTS

Principal component analyses

The first three components resulted from the analysis of 21 size-and-shape variables (Table 1) accounting for 86.6% of the total variance in the data. Scores of 16 variables (76.2%) are high or moderate (> 0.6), indicating that PC1 (79.7% of the total variance) is influenced by size-related variation. PC1 partially separates groups 1 and 2. Combination of PC1 and PC3 (2.7%) allows for complete separation between the groups. There is a large overlap between populations of each group labelled by locality. No further separation occurred on subsequent components.

The first three components yielded by the analysis of log shape variables account for 63.7% of the total variance in the data. The total variance on the shape data decreased compared with size-and-shape. The decrease (67.2%) is attributed to the residual log size that was removed by the size-correction procedure. Combination of PC1 and PC2 separates the groups with a small overlap (Fig. 2). As in the previous analysis, PC1 (47.0%) primarily separates the two groups. It has six high or moderate positive loadings and five high or moderate negative loadings. PC2 (11.3%) contrasts length of pygidial shield vs. the measurements of idiosoma and propodosomal shield.

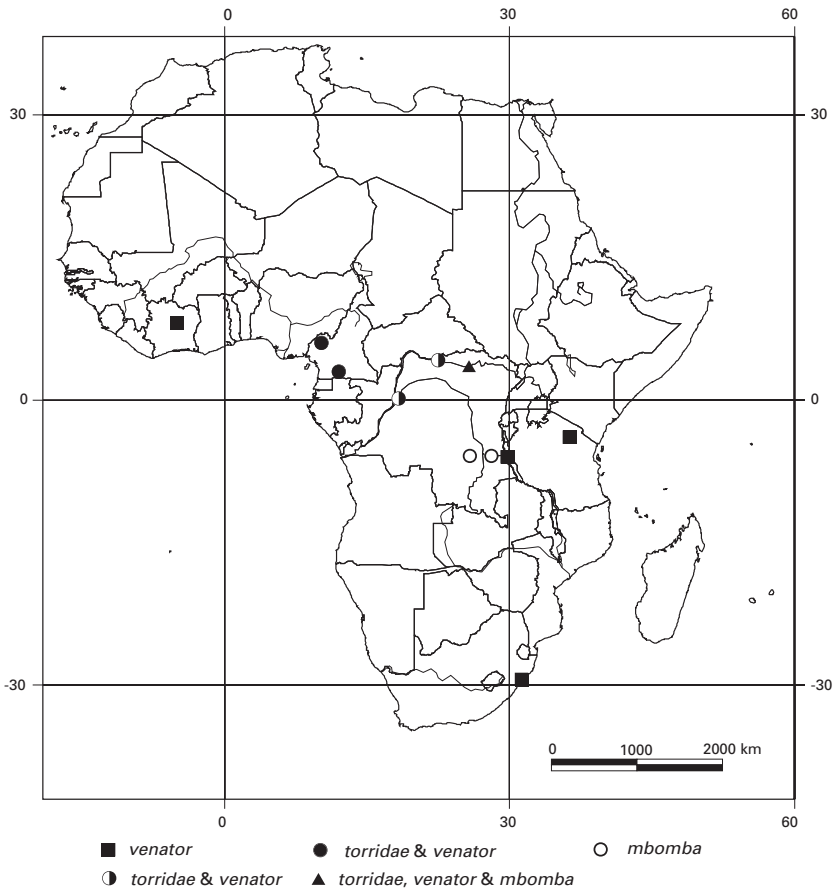

Figure 1. Collection sites of three *Cheletophyes* species associated with *Xylocopa torrida* and *X. nigrita*.

Within-group size-and-shape variation in *C. venator s.s.* from Sangmélima (Cameroon) was extensive compared to other populations. Little geographical effect in size-and-shape variation was detected for the South African population of *C. venator s.s.* Inspection of shape data showed that nearly all of these differences are size-related, although substantial shape differences were found between two populations of putative *C. torridae* from Eala (Congo) and Sangmélima.

Both size-and-shape and shape analyses confirmed our a priori assessment that mites from *X. torrida* and *X. nigrita* represent two sibling species. However, we give neither a list of variables contributing to the group separation nor other important details for the above analyses. The differences between groups will be described using a CVA, another multivariate technique focusing on prediction and description of group membership. Figure 2A and B show that almost all of the discriminatory power of log measurements is contained in log shape, justifying the use of log raw data in further calculations.

VARIABLE SELECTION

CVA of the 21 log size-and-shape variables (Table 1) resulted in one significant canonical function, indicating that the function is discriminating among the groups. Box's M statistic indicates that the covariance matrices differ between groups ($P = 0.006$), violating an important assumption of CVA, although many researchers (e.g. Hair *et al*., 1998) believe that CVA can be robust even when this assumption is violated. Although all specimens were correctly classified, one specimen (0.8%) was misclassified in jackknife cross-validation, indicating upward bias of the canonical function. This may be a result of either incorrect original grouping or the presence of variables that do not contribute substantially to the intergroup differences (Huberty, 1994). In the latter case, by forming a function of only a few predictors, and omitting redundant variables or variables that introduce 'noise' into the model, a canonical function can be formed that maximizes the separation of the groups on the discriminant score. Three methods of variable selection (see Material and Methods) were employed to identify and eliminate such variables.

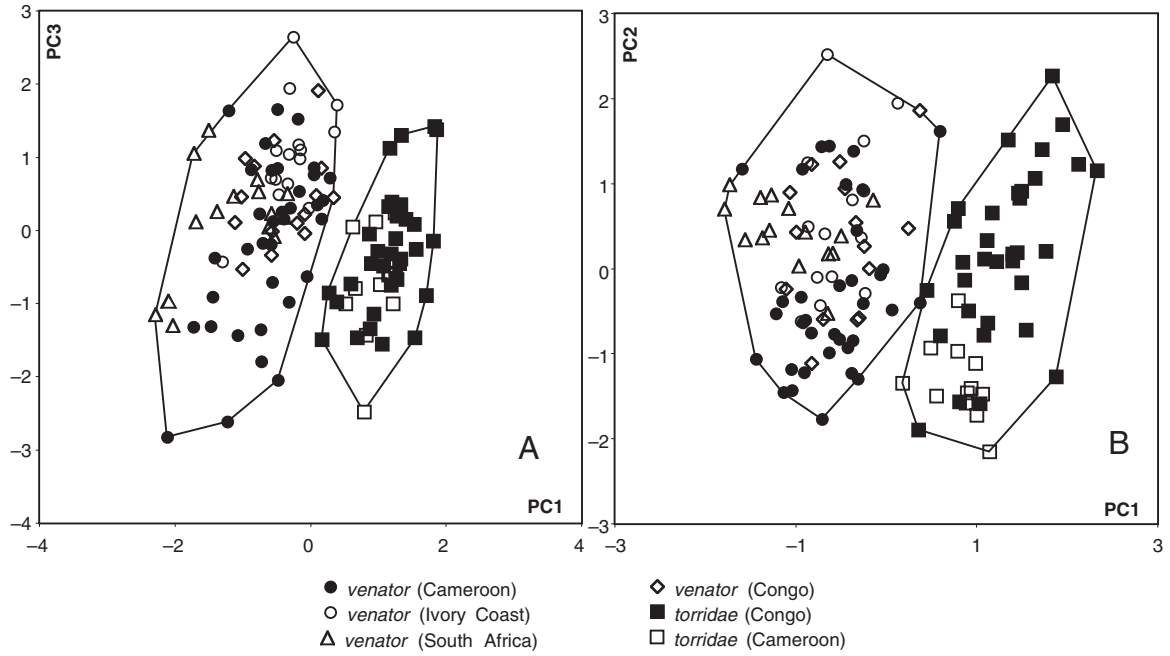

Figure 2. Plots of principal component scores (PC1 against PC2) of the 21-variable data set. A, size-and-shape; B, shape.

Figure 3 shows that data reduction based on iterative elimination of variables (where one variable with the lowest absolute loading is eliminated in each iteration) negatively affects classification accuracy in the 21-variable dataset. The misclassification rate increases substantially in all subsets of predictors. The internal hit ratio is smaller than the hit ratio estimated by the jackknife resampling, indicating positive bias of the former estimator (except for 3–2 variable subsets). Stepwise canonical variates analysis yielded a five-variable subset (length of propodosomal shield, *sci*, *d2*, *l2*, and width of pygidial shield) with 100 and 99.2% classification accuracy estimated by the analysis and jackknife sampling, respectively.

The results of the best-subset analysis (Table 2) suggest that both previous methods of data reduction failed to find the optimal subsets of discriminators. Classification rules developed by this method have fewer predictors and higher accuracy compared to the two aforementioned methods. Three subsets of size 3 and 48 subsets of size 4 were found (Table 2). We selected a single, four-variable subset as the final model for the following reasons: (1) the presence of variables *l2* and *l4*, which were proven to be easy to accurately measure; (2) two remaining variables should be present in most other subsets; and (3) the covariance matrices do not differ between groups (see Box's M statistic in Table 2; this is an assumption of discriminant analysis).

The classification accuracy of the selected subset and all other subsets found by the above analyses (Table 2) was estimated by jackknife resampling, a method based on iteratively removing one observation from the dataset and then classifying that specimen based on CVA of the remaining data (Huberty, 1994). Although the resulting error rate is less than that based on all observations, it can still be positively biased. Below we give a detailed description of the selected model and assess its prediction accuracy using an external sample.

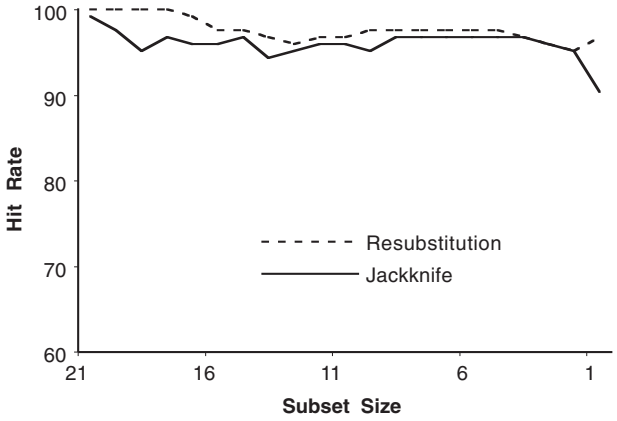

Figure 3. Variable selection based on discriminant loadings of shape data. Analysis classification accuracy is estimated by jackknife resampling.

DEVELOPING A CLASSIFICATION RULE

CVA on the four-variable subset produced a single, highly significant ($P < 0.001$) function that displays a

Table 2. Three to four variable subsets found by the best-subset analysis. All subsets have 100% classification accuracy in internal validation and jackknife resampling. The subset selected as the final model is underlined

Subset size	Box's M *P*	Variables			
3	0.001	gnathosomal length	*d1*	*l4*	
3	0.024	propodosomal shield, length	*sci*	*d2*	
3	0.240	propodosomal shield, length	*d2*	*l4*	
4	0.059	gnathosomal length	*l2*	*l4*	pygidial shield, width
4	0.084	gnathosomal length	*l1*	*l2*	*l4*
4	0.005	gnathosomal length	*d1*	*d5*	*l4*
4	0.000	gnathosomal length	*d1*	*l2*	*l4*
4	0.001	gnathosomal length	*d1*	*l4*	pygidial shield, length
4	0.005	gnathosomal length	*d1*	*N2*	*l4*
4	0.211	idiosoma, length	propodosomal shield, length	*d2*	*l4*
4	0.039	idiosoma, length	propodosomal shield, length	*sci*	*d2*
4	0.061	idiosoma, width	propodosomal shield, length	*N1*	*l2*
4	0.076	idiosoma, width	*l2*	*l4*	pygidial shield, width
4	0.069	propodosomal shield, length	*ve*	*d1*	*d2*
4	0.244	propodosomal shield, length	*ve*	*d5*	*l2*
4	0.014	propodosomal shield, length	*sci*	*d1*	*d2*
4	0.066	propodosomal shield, length	*sci*	*d2*	*l4*
4	0.048	propodosomal shield, length	*sci*	*d2*	*l1*
4	0.043	propodosomal shield, length	*sci*	*d2*	*d3*
4	0.025	propodosomal shield, length	*sci*	*d2*	*d5*
4	0.024	propodosomal shield, length	*sci*	*d2*	*l3*
4	0.022	propodosomal shield, length	*sci*	*d2*	*l2*
4	0.011	propodosomal shield, length	*sci*	*d2*	pygidial shield, length
4	0.015	propodosomal shield, length	*sci*	*N1*	*l2*
4	0.006	propodosomal shield, length	*sci*	*N2*	*d2*
4	0.036	propodosomal shield, length	*sce*	*N1*	*l2*
4	0.037	propodosomal shield, length	*N2*	*d2*	*l4*
4	0.195	propodosomal shield, length	*N1*	*d2*	*l1*
4	0.095	propodosomal shield, length	*N1*	*l1*	*l2*
4	0.061	propodosomal shield, length	*N1*	*l2*	*l4*
4	0.040	propodosomal shield, length	*l2*	*l4*	pygidial shield, width
4	0.400	propodosomal shield, length	*l1*	*l2*	pygidial shield, width
4	0.015	propodosomal shield, length	*l1*	*l2*	pygidial shield, length
4	0.359	propodosomal shield, length	*h*	*N1*	*l1*
4	0.003	propodosomal shield, length	gnathosomal length	*d1*	*d2*
4	0.062	propodosomal shield, length	gnathosomal length	*l1*	*l2*
4	0.048	propodosomal shield, length	gnathosomal length	*N1*	*l2*
4	0.152	propodosomal shield, length	*d5*	*l1*	*l2*
4	0.079	propodosomal shield, length	*d5*	*l2*	*l4*
4	0.292	propodosomal shield, length	*d2*	*d3*	*l4*
4	0.336	propodosomal shield, length	*d2*	*d5*	*l2*
4	0.165	propodosomal shield, length	*d2*	*l2*	*l4*
4	0.036	propodosomal shield, length	*d1*	*d2*	*l1*
4	0.014	propodosomal shield, length	*d1*	*d2*	*d5*
4	0.009	propodosomal shield, length	*d1*	*d2*	*l4*
4	0.013	propodosomal shield, length	*d1*	*l1*	*l2*
4	0.003	propodosomal shield, length	*d1*	*l2*	pygidial shield, width
4	0.006	propodosomal shield, length	*d1*	*l3*	*l4*
4	0.001	propodosomal shield, length	*d1*	*l4*	pygidial shield, length
4	0.015	propodosomal shield, length	*d1*	*N1*	*l2*
4	0.029	*sci*	*l2*	*l4*	pygidial shield, width

canonical correlation of 0.891. Thus, 79.4% of the variance in the dependent variable can be accounted for by this model. Box's M-test showed that the assumption of CVA about equality of covariance matrices is met ($P = 0.165$). All four variables passed the tolerance test. The unstandardized discriminant coefficients (Table 3) will be used to calculate the discriminant Z scores for classification of unknown specimens. Discriminant loadings ordered from highest to lowest by the absolute size of loadings, group centroids and cutting score are also reported in Table 3. Values of the loadings indicate that their respective variables substantially contribute to the group discrimination, where the CV is a clear contrast of *l2, d2*, and *l4* with the variable, length of propodosomal shield (Table 3). All specimens were correctly classified in resubstitution, jackknife resampling, and external validation ($N = 100$) indicating that results are highly significant and the model provides the ability to allocate an unknown specimen to one of these species with a high degree of confidence. The classification accuracy of the model substantially exceeds that expected by chance as evidenced by the proportional chance criterion (53.9%), maximum chance criterion (64.0%), and maximum chance criterion threshold value (64.0*1.25 = 80.0%).

Logistic regression

The overall model test, −2 Log Likelihood, is highly significant ($P < 0.001$), rejecting the null hypothesis that none of the independent variables are linearly related to the log odds of the dependent variable being equal to 1 (*P. torridae*). A good assessment of model fit, the Hosmer–Lemeshow test, indicates by nonsignificant chi-square value (0.291, d.f. = 8, $P = 1.000$) that there are no differences between the observed and predicted classifications. The estimated coefficients and the constant of the model were evaluated using the Wald statistic and the log-likelihood test (Table 4). The former shows that the logit coefficient for variable *l4* and the constant are significant, and while the coefficient for *l2* is insignificant, it approaches significance at the 0.05 level ($P = 0.093$). The log-likelihood test evaluates all these parameters as significant. For large logit coefficients, as in this case, standard error is inflated, lowering the Wald statistic and leading to Type II errors (Menard, 2001). Also, the Wald statistic is sensitive to violations of the large-sample assumption of logistic regression. The overall classification accuracy for the model is very high, 97.6% for the analysis and 100% for the holdout samples. One specimen of *C. venator* was misclassified as *C. torridae*, and two specimens of *C. torridae* were misclassified as *C. venator*. These results are essentially the same as for CVA conducted on the same data (not reported here).

Table 3. Loadings and unstandardized coefficients of canonical function obtained by 125 × 4 CVA. Variables ordered by absolute size of correlation within function. Centroids for groups 1 (*venator*) and 2 (*torridae*) are –1.458, 2.593, respectively

Variable	Loadings	Unstandardized coefficients
l2	0.778	7.341
d2	0.719	4.690
l4	0.706	3.514
propodosomal	0.121	–9.622
shield, length	Constant	–13.842
	Critical value	1.13464

Use of the models

In order to assign an unknown specimen to either *C. venator* or *C. torridae,* two alternative models developed by CVA and logistic regression can be used. The model developed by CVA requires measuring four variables, whereas the model derived from a logistic regression analysis has only two variables. Classification accuracy for both models is 100% when applied to a new sample ($N = 100$); however, the logistic regression model misclassified 2.4% specimens in the analysis sample.

Each of four measurements for the CVA model is converted to a natural logarithm and multiplied by the appropriate set of corresponding unstandardized coefficients (Table 3). These products and the constant are added to give the canonical variate value (CV). If the CV is less than the critical value (Table 3), the

Table 4. Logistic Regression coefficients, Standard Errors (SE), the Wald statistic, the log-likelihood test, and the corresponding significance level tests. df, degrees of freedom; Exp (B), odds ratio of the row covariate with the dependent; LL, Log Likelihood

Variable	Coefficient	SE	Wald	df	*P*	Model LL	Change in −2 LL	*P*	Exp (B)
l2	43.484	25.864	2.827	1	0.093	–12.300	11.583	0.001	7.6739E + 18
l4	40.512	18.523	4.783	1	0.029	–11.037	9.057	0.003	3.9264E + 17
Constant	–348.043	138.422	6.322	1	0.012				

specimen is classified as *C. venator*, otherwise as *C. torridae*. A Javascript application that performs the calculations is available online at http://insects.ummz.lsa.umich.edu/beemites/Morphometrics/Cheletophyes.htm.

Classification based on the two-variable logistic regression model is as follows:

$$P(C.\ torridae) = \mathrm{Exp}(-348.043 + 43.484*l2 + 40.512*l4)/(1 + \mathrm{Exp}(-348.043 + 43.484*l2 + 40.512*l4)),$$

where P is the probability of an unknown specimen being *C. torridae*. The numbers in the equation are coefficients and constant from Table 4; *l2* is the natural logarithm of the length of seta *l2* in micrometers, and *l4* is the natural logarithm of the length of seta *l4* in micrometers. The critical value is 0.5. If $P > 0.5$, the unknown specimen is predicted to be *C. torridae*, whereas if $P > 0.5$, the unknown specimen is predicted to be *C. venator*. A Javascript application that performs these calculations is available online at http://insects.ummz.lsa.umich.edu/beemites/Morphometrics/CheletophyesLR.htm.

DISCUSSION

Principal component, canonical variates and logistic regression analyses showed that *C. venator* and *C. torridae* are morphologically distinct entities, and all their differences are size-independent, i.e. probably influenced mostly by the genetic component of variation. Nevertheless, the gap between the two species does not indicate that the host effect can be completely ruled out and, thus, that the morphotypes are reproductively isolated (Claridge & Gillham, 1992). Klimov *et al*. (2004) showed that reproductively isolated mites of the genus *Sancassania* (Acari: Acaridae) collected in the field and reared in laboratory cultures exhibit small differences in shape. However, we were able to separate one species from other cryptic species using the same morphometric technique.

Our study, therefore, is only one approach in the attempt to find morphological discontinuities between populations that might provide evidence for reproductive/genetic isolation. Additional data (e.g. gene sequences, rearing experiments) will be required to test whether the mite populations are genetically distinct. The classification models developed in this paper allow for a complete discrimination between the two cryptic species *C. venator* and *C. torridae* with 100% accuracy. In several reproductively isolated cryptic species, morphological differentiation is very small, and they can only be partially separated, with an overlap in morphometric characters (Umphrey, 1996; Burks & Heraty, 2002).

The strong host specificity of *C. venator*, *C. torridae*, and *C. mbomba* suggests their close evolutionary relationships with their hosts, corroborating the hypothesis that the evolution of the mesosomal acarinaria that specifically harbour dispersing *Cheletophyes* mites may be a selective response to the presumed mutualistic association between these mites and the host bees (OConnor, 1993). The host specificity and the absence of mixed populations on *X. nigrita* can be explained by the bees' biology. Species of *Xylocopa* are solitary bees that excavate their nests in wood. The nests contain several closed cells, each with a larva and provisioned food (Gerling, Velthuis & Hefetz, 1989; Michener, 2000). Mites inhabiting these cells can only leave the cell with a newly emerged bee or its parasite. This would seem to be the primary mode of dispersal because these mites are weakly sclerotized and could survive outside the bee nest only for a short period of time. However, sympatric bee species may utilize the same substrate for contemporaneous nest construction, allowing occasional opportunities for host switching.

Cheletophyes torridae and *C. mbomba* are obligatory associates of *X. torrida*. Both species often occur on the same bee specimen, indicating that they probably inhabit the same nest. These species are morphologically well separated (see differential diagnosis of *C. mbomba* below) and probably have different ecological niches to avoid interspecific competition in the very limited space of the bee's nest cell. In contrast, the two sibling species associated with different hosts, *C. venator* from *X. nigrita* and *C. torridae* from *X. torrida*, have only subtle morphological differences.

In recent phylogenetic analyses of major lineages of *Xylocopa* based on morphology (Minckley, 1998) and molecular data (Leys, Cooper & Schwarz, 2000, 2002), the host bees, *X. nigrita* and *X. torrida*, were shown to belong to distinct clades (ranked as subgenera, *Mesotrichia* and *Afroxylocopa*, respectively) within the genus. Some of their topologies indicate that the subgenera *Mesotrichia* and *Koptortosoma* (with *Afroxylocopa* as the basal clade) are sister taxa. However, this does not mean that *X. nigrita* and *X. torrida* are sister taxa, unlike their mite associates of the genus *Cheletophyes*. This might suggest that the associations studied here are not the result of strict cospeciation of mites with their bee hosts and that host shifts commonly occur in *Cheletophyes*. These observations, with the empirical assumption that *Cheletophyes* mites depend on their host only indirectly, make it very difficult to explain the presence of strong host specificity in the analysed species of *Cheletophyes*. Host specificity can simply reflect microhabitat isolation of the hosts' nests, but this feature of the bees' biology is unknown in this case. Even if habitat isolation exists presently, we must assume it was not complete at

some past time in order to account for the cophylogenetic associations observed here.

In conclusion, coevolutionary relationships between *Cheletophyes* mites and *Xylocopa* bees are potentially very intimate. The mites and bees interact so closely that each may serve as a strong selective force on the evolution of the other (Janzen, 1980). Based on the phylogenetic relationships of the bees noted above, an Old World *Xylocopa* lineage developed the mesosomal acarinaria in female bees to transfer the predacious mites after the origin of the association. This is inferred from the fact that *X.* (*Megaxylocopa*) *frontalis* (Olivier), host to *C. panamensis*, has no such acarinaria and is only very distantly related to a clade containing all the known Old World mite hosts. *Cheletophyes* mites have adapted to such phoretic dispersal and live exclusively in the bee nests. At this point, speciation in *Cheletophyes* is more dependent on interaction with the bee hosts than to their prey, which is a possible explanation for the strong host specificity in the analysed species of *Cheletophyes*.

SYSTEMATICS

FAMILY CHEYLETIDAE LEACH, 1815
GENUS *CHELETOPHYES* OUDEMANS, 1914
SPECIES COMPLEX *VENATOR*

Mites of this species complex are characterized by the presence of three pairs of median setae (*d1, N1*, and *N2*) on the propodonotal shield and a well-developed pygidial shield bearing setae *d5* (nomenclature of idiosomal setae adopted from Fain, 1979). The first character is shared with *C. panamensis*, but its pygidial shield is weakly developed and devoid of setae. All other species of the genus bear two pairs of setae on the propodonotal shields. This complex includes three species: *C. venator, C. torridae* sp. nov., and *C. mbomba* sp. nov.

CHELETOPHYES VENATOR (VITZTHUM, 1920) (FIG. 4)

Cheletes venator Vitzthum, 1920: 2, figures 1–3.

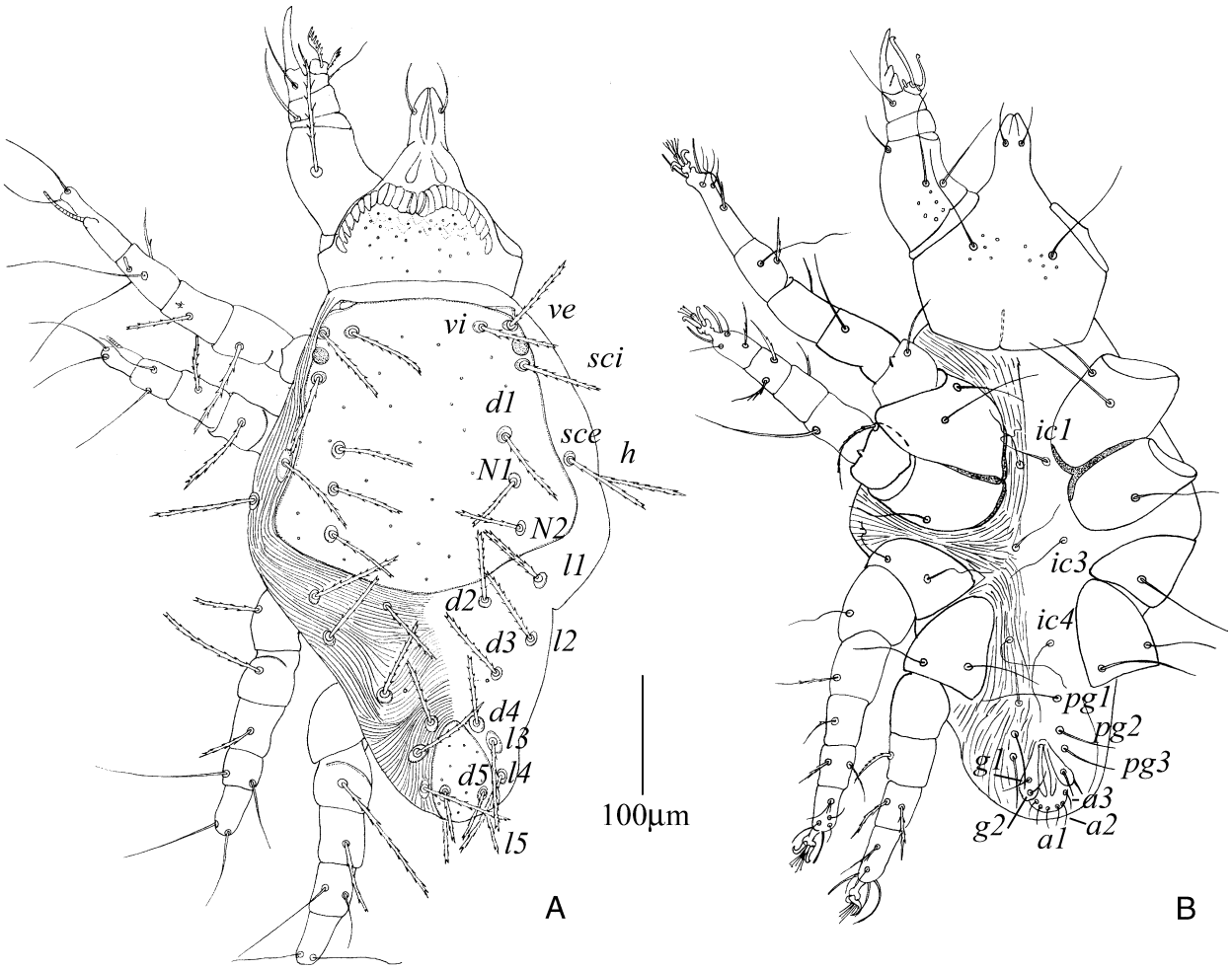

Figure 4. *Cheletophyes venator* (Vitzthum, 1920) female. A, dorsal view; B, ventral view.

Cheyletus venator: Volgin, 1969: 119, fgures 111, 112; Summers & Price, 1970: 31; Gerson, Fain & Smiley, 1999: 55.

Cheletophyes venator: Fain & Bochkov, 2001b: 84.

Cheletophyes aurorae Haitlinger, 2000: 85, figures 1–7, **syn. nov.**

Cheletophyes Vitzthumi (non Oudemans, 1914) Vitzthum, 1920: 6, figure 4.

Female (Fig. 4, see Table 1 for measurements). Gnathosoma 2 times shorter than idiosoma. Peritremes M-shaped, with 12–14 pairs of unequal segments. Rostral shield weakly ornamented in anterior part. Palpal femur 55–80 long, 40–65 wide. Dorsal seta of palpal femur 55–95 long, barbed; other palpal setae filiform. Palpal claw with 2 basal teeth. Outer comb-like seta of palpal tarsus with 6–8 tines, inner comb-like seta with 4–5 tines. Idiosoma rhomboid, its anterior half covered by propodonotal shield. Propodonotal shield devoid of ornamentation; length approximately equal to width, bearing setae *vi, ve, sci*, and 3 pairs of median setae *d1, N1*, and *N2*. Setae *sce* situated off shield. Eyes large. Pygidial shield well-developed, devoid of ornamentation, its length/width ratio 1.2–1.3 : 1, bearing setae *d5* and *l5*. All dorsal setae roughly barbed and subequal in length, except for relatively short *l5* (28–35); all ventral setae nude, filiform, situated on small, poorly sclerotized platelets. There are 3 pairs of cupules, and full set of dorsal and ventral setae, *d1-d5, l1-l5, ic1, ic3, ic4, pg1-pg3, g1, g2*, and *a1-a3*. Legs. Tarsi I, excluding pretarsi, 1.5 times longer than solenidion *omega I*. Guard seta of solenidion absent. Leg setation I–IV (solenidia): trochanters 1-1-2-1, femora 2-2-2-1, genua 2(1)-2-2-2, tibiae 5(1)-4-4-4, tarsi 8(1)-7(1)-7–7. Shapes of leg setae as in Figure 4.

Material. Thirty-one females (BMOC 03-0601-006), CAMEROON: Nord Prov., Sangmélima, Fulasi, *ex Xylocopa nigrita,* mesosomal acarinarium, 1.iii.1920 (*Evans*) (UMMZ); 5 females (BMOC 03-0601-005), same data, 18.i.1924 (UMMZ); 18 females (BMOC 03-0601-007), same data, 25.i.1924 (UMMZ); 15 females (BMOC 03-0630-003), CAMEROON: Ouest Prov., Bambui, Bamenda, *ex X. nigrita*, mesosomal acarinarium, 9.vii.1966 (*Michener*) (KU); 5 females (BMOC 03-0630-002), TANZANIA: Arusha Reg., Karalu, 5 km N Gibb's Farm, 1750 m, *ex X. nigrita*, mesosomal acarinarium, 25.xi.2001 (*Brzoska*) (KU); 10 females (BMOC 03-1005-001), TANZANIA: Kigoma Reg., Kigoma Distr., Mahale Mountains National Park, Mkala Bay, 6.6 km S Park Headquarters, elev. 773–780 m, lakeshore, 06°05′41″S, 029°43′54″E, *ex X. nigrita* (2003–0016), mesosomal acarinarium, 8.viii.2003 (*OConnor*) (UMMZ); 25 females (BMOC 03-0830-005), CONGO: Bas-Uele Prov., Bambesa, *ex X. nigrita* (coll. number 12.131), mesosomal acarinarium, no date (*Vrydagh*) (IRSNB); 25 females (BMOC 03-0830-006), IVORY COAST, *ex X. nigrita*, mesosomal acarinarium (IRSNB); 25 females (BMOC 03-0630-004), SOUTH AFRICA: KwaZulu-Natal, Salt Rock, 29 mi N. Durban, *ex X. nigrita*, mesosomal acarinarium, 25.ii.1967 (*Michener*) (KU).

Remark. A single male and nymph of this species were described from the nest of *X. nigrita* from Amani, Tanzania (formerly Tanganyika) (Vitzthum, 1920). All relevant characters of the male resemble those of females we collected from *X. nigrita*. Because we found only one species of *Cheletophyes* on *X. nigrita*, we assume that this is *C. venator*.

Both logistic regression and canonical variates models show that *Cheletophyes aurorae* is a junior synonym of *C. venator*. Measurements for the models were acquired from Haitlinger's (2003: 87) digitized figure 1 and are as follows: *l2* 50, *d2* 43, *l4* 48, and length of propodosomal shield 174.

CHELETOPHYES TORRIDAE BOCHKOV, KLIMOV & OCONNOR **SP. NOV.**

Female (holotype, see Table 1 for measurements). Gnathosoma 2 times shorter than idiosoma. Peritremes M-shaped, with 14 pairs of unequal segments. Rostral shield weakly ornamented in anterior part. Palpal femur 75 long and 57 wide. Dorsal seta of palpal femur 85 long, barbed; other palpal setae filiform. Palpal claw with 2 basal teeth. Outer comb-like seta of palpal tarsus with 8 tines, inner comb-like seta with 4–5 tines. Other characters as in previous species.

Differential diagnosis. This species is distinguishable from the closely related *C. venator* only by group means of some measurements (Table 1). The best univariate discriminators are length of setae *l2*: 64–88 (77.1 ± 5.22) in *C. torridae* and 44–70 (57.2 ± 5.59) in *C. venator*; and length of setae *l4*: 59–77 (67.2 ± 4.44) in *C. torridae* and 40–62 (52.0 ± 5.08) in *C. venator*. To separate these two species use either the four-variable model developed by CVA or the two-variable model developed by logistic regression. See details in the section 'Use of the models' above.

Etymology. The species name is derived from the specific name of the host and is a noun in the genitive case.

Type material. *Holotype,* female (BMOC 03-0601-009), CAMEROON: Nord Prov., Sangmelima, Fulasi, *ex X. torrida*, mesosomal acarinarium, 1.iv.1920 (*Evans*) (UMMZ). *Paratypes*, 8 females, same data; 5 females (BMOC 03-0601-008), same data; 2 females (BMOC 90-1212-013); same data (UMMZ – 12 females; IRSNB – 1, KU – 1, MRAC – 1, ZIN – 1).

Additional material. Two females (BMOC 03-0630-001), CAMEROON: Ouest Prov., Bambui, Bamenda, *ex X. torrida*, mesosomal acarinarium, 1.ix.1965 (*Michener*) (KU); 8 females (BMOC 03-0829-003), CONGO: Bas-Uele Prov., Bambesa, *ex X. torrida* (coll. number 12.292), mesosomal acarinarium, 26.i.1939 (*Vrydagh*) (IRSNB); 2 females (BMOC 03-0829-001), the same data, *ex X. torrida* (coll. number 12. 284), 14.i.1939 (*Vrydagh*) (IRSNB); 4 females (BMOC 03-0829-002), same data, *ex X. torrida* (coll. number 12. 429), 20–22.iii.1939 (*Vrydagh*) (IRSNB); 25 females (BMOC 03-0830-004), CONGO: Nord-Ubangi Prov., Uele, Tukovo, *ex X. torrida*, mesosomal acarinarium, vii.1937 (*Vrydagh*) (MRAC); 19 females (BMOC 03-0830-002), CONGO: Equateur Prov., Eala, *ex X. torrida*, mesosomal acarinarium, iii.1932 (*Bredo*) (MRAC); 17 females (BMOC 03-0924-001), CONGO, multiple specimens of *X. torrida*, mesosomal acarinarium (MRAC); 11 females (BMOC 03-0924-002), CONGO, ex multiple specimens of *X. torrida*, mesosomal acarinarium (MRAC); 4 females (BMOC 03-0924-003), CONGO, multiple specimens of *X. torrida*, mesosomal acarinarium (MRAC).

CHELETOPHYES MBOMBA BOCHKOV, KLIMOV & OCONNOR **SP. NOV.**
(FIG. 5)

Female (holotype, Fig. 5, see Table 1 for measurements). Gnathosoma 2.2 times shorter than idiosoma. Peritremes M-shaped, with 12–13 pairs of unequal segments. Rostral shield weakly ornamented in anterior part. Palpal femur 55 long, 37 wide. Dorsal seta of palpal femur 65 long, barbed; other palpal setae filiform. Palpal claw with 3 (2–3 in paratypes) basal teeth. Outer comb-like seta of palpal tarsus with 8–9 tines, inner comb-like seta with 5–6 tines. Idiosoma rhomboid, its anterior half covered by propodosomal shield. Propodosomal shield covered with fine reticulate ornamentation; its length/width ratio 1 : 1, bearing setae *vi, ve, sci*, and 3 pairs of median setae, *d1, N1*, and *N2*. Setae *sce* situated off shield. Eyes large. Pygidial shield very large, without ornamentation, its length/width ratio 1 : 1 (1–1.1 : 1 in paratypes), bearing setae *l4*, *d5* and *l5*. All dorsal setae situated on small, poorly sclerotized platelets, roughly barbed and subequal in length except for relatively short *l5* (33). Legs. Tarsi I, excluding pretarsi, 1.7 times longer than solenidion *omega I*. Other characters as in other species of the complex *venator*.

Type material. *Holotype*, female (BMOC 03-0829-003), CONGO: Bas-Uele Prov., Bambesa, *ex Xylocopa torrida* (coll. number 12.292), mesosomal acarinarium, 26.i.1939 (*Vrydagh*) (MRAC). *Paratypes*, 10 females, same data (IRSNB – 8 females, UMMZ –1, ZIN – 1).

Figure 5. ***Cheletophyes mbomba*** **sp. nov.** Female, dorsal view.

Additional material. Twenty-eight females (BMOC 03-0830-002), CONGO: Eala, ex *Xylocopa torrida*, mesosomal acarinarium, iii.1932 (*Bredo*) (MRAC); 16 females (BMOC 03-0830-004), CONGO: Equateur Prov., Uele, Tukovo, *ex X. torrida*, mesosomal acarinarium, vii.1937 (*Vrydagh*) (MRAC); 10 females (BMOC 03-0830-001), CONGO: Tanganyika Prov., Nyunzu, *ex X. torrida*, mesosomal acarinarium, i–ii.1934 (*De Saeger*) (MRAC); 21 females (BMOC 03-0830-003), CONGO: Kwango Prov., Zone de Kapanga, Lulua, *ex X. torrida*, mesosomal acarinarium, ix.1932 (*Overlaert*) (MRAC).

Etymology. The name of this predaceous mite is derived from the name of the African god Mbomba, master of life and death of the Mongo people occupying the Congolese Central Basin, a noun in apposition.

Differential diagnosis. This species clearly differs from the two other species of the complex *venator* by the reticulate ornamentation of the propodonotal shield, the localization of setae *l4* on the pygidial shield, and the large size of the pygidial shield (70–92 length and 66–77 width). In *C. venator* and *C. torridae*, the propodonotal shield is devoid of ornamentation, setae *l4* are situated off the pygidial shield,

the pygidial shield is 37–66 long and 33–51 wide in *C. venator*, and 44–70 long and 33–48 wide in *C. torridae*.

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