Pergamon

International Journal for Parasitology, Vol. 25, No. 12, pp. 1401–1411, 1995
Australian Society for Parasitology
Elsevier Science Ltd
Printed in Great Britain

0020-7519(95)00058-5

0020-7519/95 $9.50 + 0.00

Morphological and Morphometric Study of Crustacean Parasites within the Genus *Lernaeocera*

P. A. VAN DAMME and F. OLLEVIER

Laboratory of Ecology and Aquaculture, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium

(Received 9 June 1994; accepted 4 April 1995)

Abstract—Van Damme P. A. & Ollevier F. 1995. Morphological and morphometric study of crustacean parasites within the genus *Lernaeocera*. *International Journal for Parasitology* 25: 1401–1411. Two species of *Lernaeocera* are present in the southeastern North Sea. *Lernaeocera lusci* infects bib *Trisopterus luscus*, dragonet *Callionymus lyra* and sand goby *Pomatoschistus minutus*. *L. minuta* is a junior synonym of *L. lusci*. The second valid species, *L. branchialis*, infects whiting *Merlangius merlangus*. The two species can be morphologically separated by the antennary processes, which are present in *L. lusci* and absent in *L. branchialis*. Discriminant functions allow complete separation between *L. lusci* and *L. branchialis*. There is high intraspecific, host-dependent variability within *L. lusci*. Length of *L. lusci* is significantly influenced by host size, and body form is influenced by the site of attachment of *L. lusci* on at least one host (bib). It is suggested that *L. lusci* consists of 3 forms: f. *lusci*, f. *minuta* and f. *lyra*.

Key words: Lernaeocera branchialis; *Lernaeocera lusci*; morphometry; copepoda.

INTRODUCTION

The post-metamorphosis females of *Lernaeocera* species are characterized by a thoracic holdfast, normally consisting of 1 dorsal branch and 2 lateral branches. These structures are used for deep penetration in the definitive host tissues, hence the name mesoparasites for the members of this genus (Kabata, 1979). The genito-abdominal part, however, is external to the host.

The literature on the genus *Lernaeocera* contains many descriptions of taxa based on unusually shaped individuals. Kabata (1979), in the most recent thorough investigation of the genus, argued that only a small number of *Lernaeocera* species are valid. The same author also presents strong evidence that *Lernaeocera lusci* and *L. branchialis* are 2 separate species, the absence (*L. branchialis*) or presence (*L. lusci*) of antennary processes being the main distinguishing characteristic (Slinn, 1970; Kabata, 1979). Moreover, Tirard (1991), using enzyme electrophoresis, found that no gene flow occurred between sympatric populations of *L. lusci* and *L. branchialis*.

The validity of a third species, *L. minuta* (Scott, 1900), still requires confirmation (Kabata, 1979). The general appearance and the structure of the appendages of *L. minuta* are morphologically very similar to those of *L. lusci*. It has therefore been doubted whether the 2 can be considered to be distinct species. The main characteristic used to distinguish *L. minuta* from *L. lusci* and *L. branchialis* is its smaller size, a criterion of doubtful taxonomic significance for parasitic crustaceans (Kabata, 1979). Indeed, if the size criterion proves to be of limited use, a need arises for alternative methods to detect appreciable differences between *L. minuta* and the other species. It is obvious that a thorough taxonomic study should precede ecological studies on the population dynamics of these parasite species. This effort is not superfluous, because correct interpreta-

tion of the results obtained by ecological studies on these parasites depends heavily on the validity of the species on which they focus.

The systematics of parasitic crustaceans are particularly difficult to study because many of their morphological structures have undergone drastic reduction. Recent methods for studying speciation within parasitic crustaceans include morphometry (Bastide-Guillaume, Douëllou, Romestand & Trilles, 1987; Tirard, 1991) and, especially, enzyme electrophoresis (Bastide-Guillaume *et al.*, 1987; Zeddam *et al.*, 1988; de Meeus, Renaud & Gabrion, 1990; Tirard, 1991). In the present paper we will call on morphometrics as a first step towards the elucidation of speciation within this genus.

During regular coastal and estuarine fish surveys in the Belgian and Dutch coastal waters conducted between 1988 and 1992, many demersal fish species were found to harbour mature females of *Lernaeocera lusci*. Bib *Trisopterus luscus*, whiting *Merlangius merlangus*, sand goby *Pomatoschistus minutus*, dragonet *Callionymus lyra* and five-bearded rockling *Ciliata mustela* all played a more or less important role in the life-cycle of either *Lernaeocera lusci*, *L. branchialis* or *L. minuta* (cf. Van Damme & Hamerlynck, 1992; Van Damme *et al.*, 1993; Van Damme & Ollevier, 1994). As well as the central question concerning the validity of *Lernaeocera* species, the question of which parasite species infects which host species will be addressed in the present study.

The outline of this study is as follows: (1) a morphological study (by scanning electron microscopy) was carried out of the head region of parasites collected from 4 different host species (dragonet, bib, whiting, sand goby). Special attention was paid to the presence of the antennary processes. (2) The effect of host length on the size of *L. lusci* occurring on bib, dragonet and sand goby was studied. By testing the host size-dependence of parasite length, the hypothesis that parasite size is a suitable criterion to discriminate between *Lernaeocera* species is assessed. (3) The third aim is to assess the morphological similarity between *Lernaeocera* individuals on the basis of selected combinations of morphometric variables and to identify factors (host species, site of attachment) which determine the morphological variability of the valid species. The degree of interspecific and intraspecific morphological variability is studied by multivariate methods.

MATERIALS AND METHODS

Parasites from 4 demersal fish species were examined. The fish were collected with a beam trawl by means of research vessels or commercial shrimp trawlers in the Oosterschelde and along the Belgian coast. The following species were collected: sand gobies *Pomatoschistus minutus*, bib *Trisopterus luscus*, whiting *Merlangius merlangus* and dragonet *Callionymus lyra*.

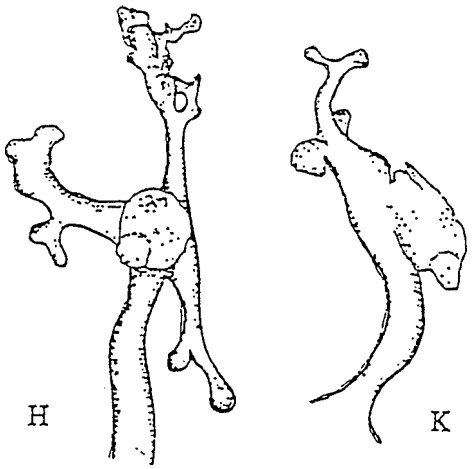

Fig. 1. Head of H-types and K-types of *Lernaeocera lusci*.

The fish were transferred to a 7% formalin solution immediately after capture. About 1 month later the fish were transferred to ethanol 70%. All parasites which were collected from one host species were grouped *a priori*. Thus, 4 source groups were distinguished: LLTL (*L. lusci* on bib), LMPM (*L. minuta* on sand goby), LLCL (*L. lusci* on dragonet) and LBMM (*L. branchialis* on whiting). The source groups contained 49, 39, 36 and 17 individuals, respectively. Group LLTL was further divided into 2 subgroups $LLTL_K$ and $LLTL_H$. The definition of these subgroups was based on the occurrence of 2 different types of *Lernaeocera lusci* on bib: the first type (K) has a well developed dorsal branch and no lateral branches, the second type (H) has both dorsal and lateral branches. The differences between the different types are summarized in Fig. 1. For *L. lusci* collected from bib there was a significant correlation between the type and the site of attachment: all K-specimens were embedded with their proximal parts completely within the gill arch tissue, while H-specimens were significantly more often embedded with their head in the posterior ridge of the gill chamber ($\chi^2 = 43.9$, $P<0.001$). Subgroups $LLTL_K$ and $LLTL_H$ contained 30 and 17 specimens, respectively. One H-type specimen was embedded in the gill arch and was excluded from the analysis. For another specimen the exact site specificity was uncertain. The number of K-parasites in *L. lusci* collected from *P. minutus* and from *C. lyra* was negligible.

The morphology of the adult female *Lernaeocera* was studied by scanning electron microscopy. Only the head region was taken into account. Particular attention was given to the presence of the antennary processes.

Total parasite length (G) was measured as in Fig. 2. The total length of the fish was measured from the tip of the

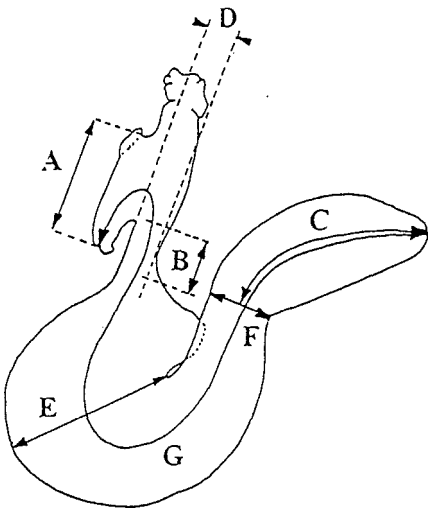

Fig. 2. Body measurements *A–G* of *Lernaeocera* spp. (A = length proboscis, B = length neck, C = length abdomen, D = width neck, E = width trunk, F = width abdomen, G = total length).

mouth to the end of the tail. Linear regression equations of the form $y = a+bx$ describing the relationship between host size (x) and parasite size (y) were calculated. The significance of the regression coefficients was tested (F-test).

Length of proboscis, length of neck, length of abdomen, width of neck, width of trunk, width of abdomen and total length were measured as in Fig. 2. Prior to further calculations the means for the variables were calculated. Subsequently, covariance analysis (ANCOVA) was used to check whether significant differences occurred between the means.

Size-effects were removed from the data matrices by a multivariate adjustment. Application of Burnaby's (1966) method, as modified by Rohlf & Bookstein (1987), to the data matrices yielded adjusted variables A'–G'.

To detect interspecific and intraspecific morphological variability of *Lernaeocera*, 4 source groups LLTL, LLCL, LMPM and LBMM were used (141 parasites). The data were subjected to a stepwise forward discriminant analysis to select a subset of variables. Then, the selected variables were subjected to a canonical discriminant analysis (CDA). Mahalanobis distances between individual observations were calculated. Subsequently, individual observations were allocated to the group for which they had the minimal Mahalanobis distance. These analyses were carried out with the raw data matrix and with the matrix containing adjusted data.

Furthermore, source groups $LLTL_K$, $LLTL_H$, LLCL and LMPM (a total of 122 individuals) were used to test the effect of host identity and site of attachment on the morphology of *L. lusci* and *L. minuta*. The data were corrected for size-effects by Burnaby's (1966) method. After that, the same procedure as described above was followed.

RESULTS

The head region of *Lernaeocera* from different host species is shown in Fig. 3. The holdfasts of the majority of individuals have 1 dorsal (Da) and 2 lateral branches (La), often of unequal length. *Lernaeocera branchialis* (definitive host: whiting) (Fig. 3A) can be clearly distinguished from all other individuals by the absence of antennary processes (Ap). The mouth of this species is at the anterior end of a short proboscis. A ringed chitinous structure, the buccal tube, distal of the mouth cone (Mt), forms the mouth opening. All parasites belonging to the species *Lernaeocera lusci* and collected from *Trisopterus luscus* and *Callionymus lyra* possess antennary processes (Ap) (Fig. 3B, C). The antennary processes consist of 2 branches which may be simple or subdivided (Fig. 3B). The mouth cone (Mt) is located at the apex of a long proboscis and appears to be longer than in *L. branchialis*. Individuals collected from dragonet and bib are morphologically indistinguishable from *L. minuta* collected from sand goby. Though the proboscis of individuals collected from sand gobies (Fig. 3D) seems to be shorter than in *L. lusci* collected from bib, the general appearance is the same: a lateral view clearly shows the antennary processes (Ap), the mouth cone (Mt) and the thoracic legs (Le).

The effect of host size on the total length of *Lernaeocera lusci* is plotted in Fig. 4. For LLTL and for LLCL a significant positive relationship was found between parasite size and host size. There was no such correlation for LMPM. The linear regression equations which describe the relationship between total host length and total parasite length are shown in Table 1.

The means of the total length of the 4 source groups (LLTL, LMPM, LLCL, LBMM) are shown in Table 2. The largest individuals were *L. branchialis* collected from whiting (mean 22 mm), followed by *L. lusci* from dragonet (mean 15 mm), *L. lusci* from bib (mean 14 mm) and *L. lusci* from sand goby (mean 10 mm). Because parasite size was strongly dependent on host size, the measurements were adjusted for parasite length by covariance analysis. The means

Table 1—Coefficients of linear regression equations of the form $y = a+bx$ describing the relationship between total parasite length (y) (mm) and fish length (x) (mm) for *Lernaeocera lusci* (from bib, dragonet) and *L. minuta* (from sand goby)

Host species	n	a	b
Bib	75	5.14	0.06***
Dragonet	53	7.84	0.04**
Sand goby	31	7.70	0.01 ns

ns = not significant; ** = $P<0.01$; *** = $P<0.001$.

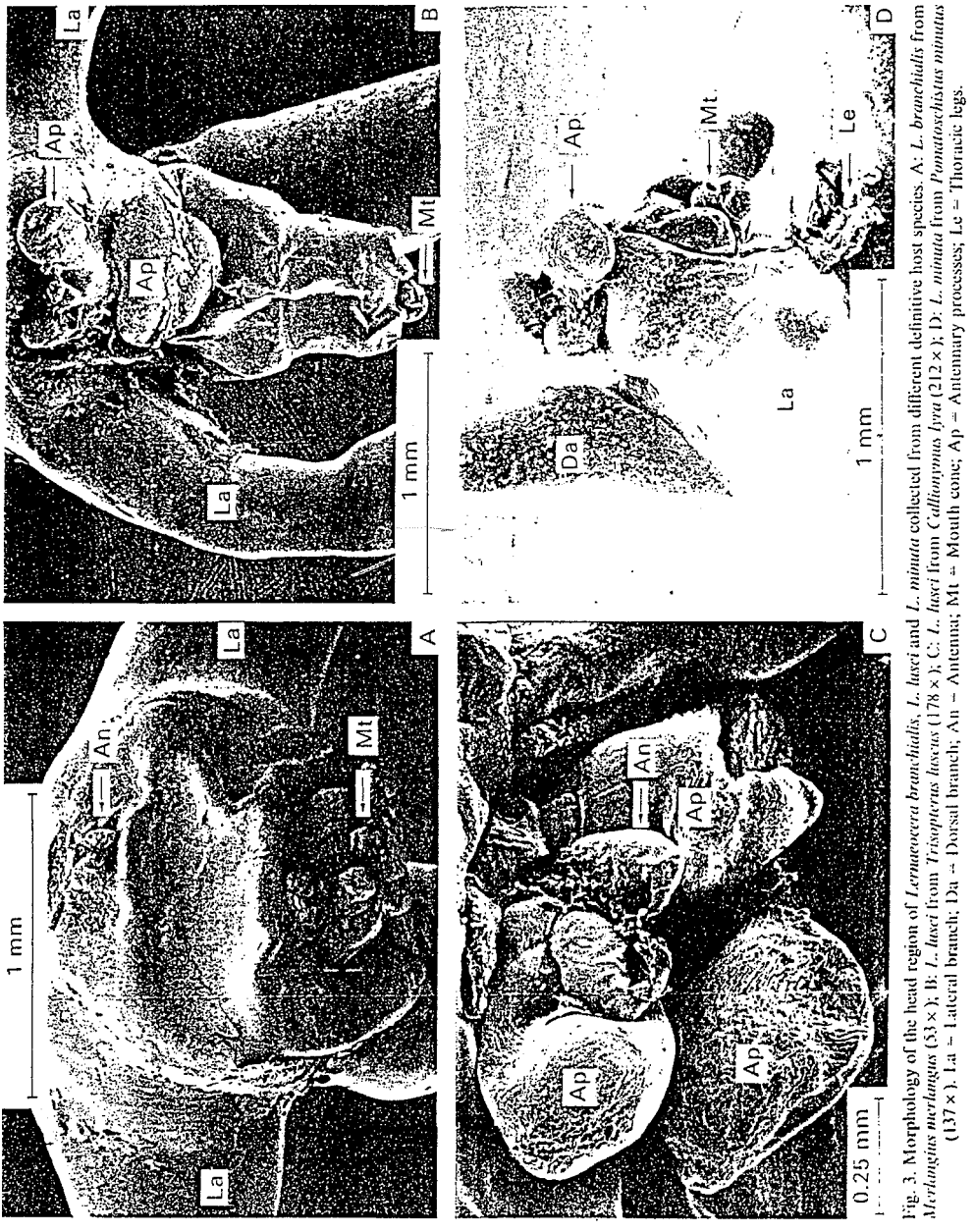

Fig. 3. Morphology of the head region of *Lernaeovera branchialis*, *L. lusci* and *L. minuta* collected from different definitive host species. A: *L. branchialis* from *Merlangius merlangus* (53×); B: *L. lusci* from *Trisopterus luscus* (178×); C: *L. lusci* from *Callionymus lyra* (212×); D: *L. minuta* from *Pomatoschistus minutus* (137×). La = Lateral branch; Da = Dorsal branch; An = Antenna; Mt = Mouth cone; Ap = Antennary processes; Le = Thoracic legs.

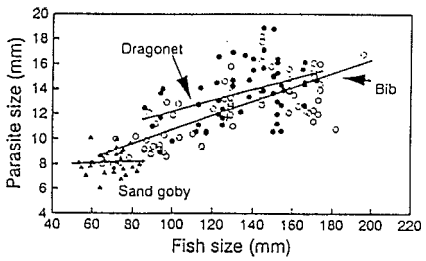

Fig. 4. The correlation between parasite size (total length) of *Lernaeocera lusci* and *L. minuta* (source groups LLTL, LLCL, LMPM) and host size (total length) for 3 different host species (sand goby, dragonet, bib).

of the adjusted variables are shown in Table 2. Significant differences (ANCOVA: $P<0.001$) in the mean adjusted values were found for all measures except abdomen length ($P>0.05$). *L. lusci* on bib had a significantly longer proboscis than *L. lusci* on dragonet and sand goby. *L. branchialis* had the shortest proboscis. *L. lusci* on dragonet was characterized by a significantly longer and broader neck than all other groups. On the other hand, this group had a very narrow trunk and a constricted abdomen. *L. lusci* on bib was characterised by significantly higher values than all other groups for these latter variables.

An *a priori* stepwise forward discriminant analysis showed that the variable neck width could be removed from the data set. Thus, only the remaining 6 variables were used in the canonical discriminant analysis. The eigenvalues for the 3 discriminant functions were 4.13, 1.33 and 0.83. The three roots accounted for 66, 21 and 13% of the explained variance, respectively. The first discriminant function was mainly determined by total length. Indeed, it was found that the first canonical root and total length were highly correlated ($r = 0.94$). Also the second discriminant function is determined mostly by total length, but the variables trunk width and abdomen width also contribute (with negative sign) to this function. Discriminant function 3 is determined mainly by variable proboscis length. A scatterplot for the first 2 discriminant functions is shown in Fig. 5A. *L. lusci* and *L. minuta* (source groups LMPM, LLTL and LLCL) and *L. branchialis* (source group LBMM) are distinctly separated along the first axis (which was highly correlated with parasite length, see above). The smaller individuals of source group LMPM are plotted to the left. Calculation of the second function allowed for some additional discrimination between source groups LLTL (characterised by high values for the variables trunk width and abdomen width) and the pool of groups LLCL and LMPM. Because the third discriminant function explained a relatively high proportion of the variance (13%), the individuals were also plotted in the plane of Roots 1 and 3 (Fig. 5B). Along the third axis some additional separation was obtained between LMPM and LBMM (both characterised by a relatively short proboscis) on the one hand, and LLCL and LLTL (both with longer proboscis) on the other hand. Calculation of Mahalanobis distances for individual cases revealed that all whiting parasites were allocated to the correct source group. Though 9% of the *L. lusci* were allocated to the wrong source group, none of these was assigned to the whiting group (Table 3). Thus, this analysis yielded 100% discrimination between *L. lusci* and *L. branchialis*.

Size correction by Burnaby's method yielded 7 adjusted variables. When stepwise forward discriminant analysis was applied to the adjusted data matrix one variable (abdomen length) was excluded from the model. Application of canonical discriminant analysis to the resulting data matrix of 6 variables and 141 cases yielded 3 axes with eigenvalues of 1.42, 1.28 and 0.29, respectively. The percentages of the total variance explained by the three eigenvectors were 48, 42 and 10%. Discriminant functions 1 and 3 were mainly determined by

Table 2—Means and S.D. of body measurements (in mm) for 4 groups of parasites (*Lernaeocera lusci* on dragonet and bib, *L. minuta* on sand goby and *L. branchialis* on whiting). All measurements except total length were adjusted for total length by covariance analysis

Host: Group:	Sand goby LMPM	Dragonet LLCL	Bib LLTL	Whiting LBMM
Length proboscis	1.34 (0.28)^{bc}	1.51 (0.42)^{b}	1.78 (0.35)^{a}	0.98 (0.28)^{c}
Length neck	3.66 (0.55)^{b}	4.23 (0.94)^{a}	3.08 (0.79)^{b}	2.84 (0.45)^{b}
Length abdomen	3.73 (0.41)^{a}	3.82 (0.46)^{a}	3.76 (0.37)^{a}	4.34 (0.40)^{a}
Width neck	0.60 (0.16)^{b}	0.78 (0.12)^{a}	0.66 (0.10)^{b}	0.62 (0.11)^{b}
Width trunk	2.39 (0.36)^{b}	1.97 (0.29)^{c}	2.67 (0.34)^{a}	2.14 (0.34)^{b}
Width abdomen	1.26 (0.25)^{b}	1.04 (0.17)^{c}	1.51 (0.24)^{a}	1.16 (0.19)^{c}
Total length	9.9 (1.2)^{c}	14.7 (2.5)^{b}	14.0 (1.7)^{b}	22.2 (3.1)^{a}

Means with the same superscript in the same row are not significantly different (Tukey test for unequal sample sizes).

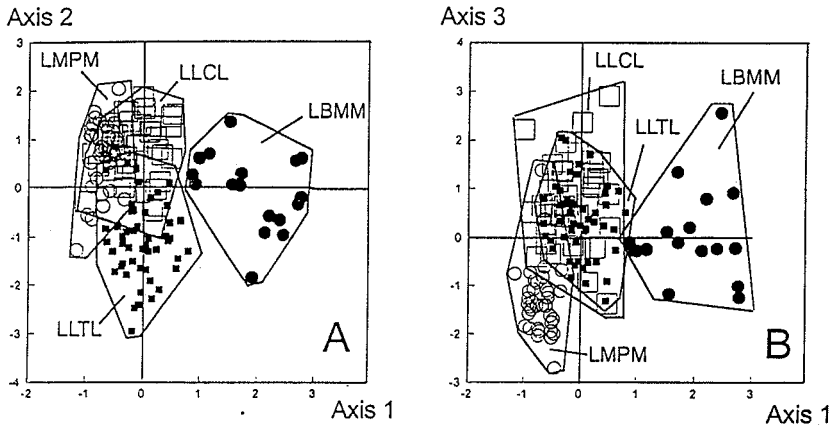

Fig. 5. Scatterplots derived from a canonical discriminant analysis on body measurements (raw data) of 4 source groups (LLTL, LLCL, LMPM, LBMM); (A) the plane formed by axes 1 and 2, (B) the plane formed by axes 1 and 3. The individuals of all groups are outlined.

proboscis length and neck length, respectively. Several variables contributed equally to discriminant function 2. The best separation between *L. branchialis* and *L. lusci* was obtained along the first and second axes (Fig. 6). The third axis gave some additional separation between LMPM on the one hand and pooled groups LLCL and LLTL on the other. Overall, 98% of all individuals were assigned to the correct species (Table 3): next to all whiting parasites 3 *L. lusci* were classified in the LBMM source group. About 21% of *L. lusci* were classified to a wrong *L. lusci* source group.

There was no significant difference in total length between parasites belonging to the K- or H-type ($F = 4.2$, $P > 0.05$) (Table 4). The means of the adjusted measurements of source groups LLCL,

Table 3—Discriminant analyses on body measurements (*A–G*) of *Lernaeocera lusci*, *L. minuta* and *L. branchialis*: classification matrix displaying the number out of 141 parasites which were allocated to the groups for which they have the minimum Mahalanobis distance. (A) Raw data matrix, (B) data for source groups LMPM, LLTL, LLCL and LBMM, adjusted for size-effects by Burnaby's method, (C) data for source groups $LLTL_H$, $LLTL_K$, LLCL, LMPM, adjusted for size-effects by Burnaby's method

Source group	% correctly classified	Number allocated to group				Total
		LMPM	LLCL	LLTL	LBMM	
A						
LMPM	92	33	1	2	0	36
LLCL	90	2	35	2	0	39
LLTL	94	0	3	46	0	49
LBMM	100	0	0	0	17	17
Sum	93	35	39	50	17	141
B						
LMPM	72	26	2	7	1	36
LLCL	87	3	34	1	1	39
LLTL	74	11	1	36	1	49
LBMM	100	0	0	0	17	17
Sum	80	40	37	44	20	141
C						
LMPM	78	28	3	3	2	36
LLCL	87	4	34	0	1	39
$LLTL_K$	71	4	0	12	1	17
$LLTL_H$	90	2	1	0	27	30
Sum	83	38	38	15	31	122

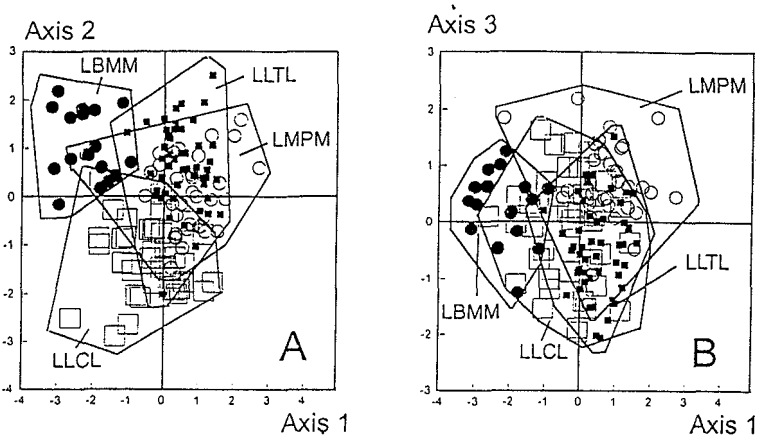

Fig. 6. Scatterplots derived from a canonical discriminant analysis on body measurements adjusted for size-effects by Burnaby's method, for 4 source groups (LLTL, LLCL, LMPM, LBMM); (A) the plane formed by axes 1 and 2, (B) the plane formed by axes 1 and 3. The individuals of all groups are outlined.

LMPM, $LLTL_K$ and $LLTL_H$, which were corrected for total length by covariance analysis, are shown in Table 4. There were significant differences between source groups $LLTL_H$ and $LLTL_K$ for 2 out of 6 variables: the neck was longer in $LLTL_H$, whereas the proboscis was longer in $LLTL_K$.

All variables were selected by stepwise forward discriminant analysis and were subjected to canonical discriminant analysis. Roots 1 ($\lambda_1 = 2.02$) and 2 ($\lambda_2 = 0.92$) accounted together for 97% of the variance. Discriminant functions 1 and 2 were mainly determined by the variables proboscis length and neck length. Individuals belonging to source groups $LLTL_K$ and $LLTL_H$ were separated along the first and second axes (Fig. 7). The good separation between these 2 groups is also shown by the classification matrix (Table 3): 71% of the K-type parasites were assigned to the correct source group (a single individual was assigned to $LLTL_H$), whereas 90% of the H-type parasites were classified in the correct source group (no individuals were assigned to $LLTL_K$).

DISCUSSION

Species belonging to the genus *Lernaeocera* are characterised by a high degree of morphological variability. Their size and shape is probably determined by an as yet unknown combination of genetic and environmental factors, the latter including host identity, host size and site of attachment. It is therefore not surprising that taxonomists accept only few characteristics as valid recognition marks for *Lernaeocera* species (Kabata, 1979; Eiras & Santos, 1990). Kabata's (1979) key to the species of *Lernaeocera* allows for distinguishing *L. lusci* from *L. branchialis*. The most important difference is the presence of the antennary processes in *L. lusci* (absent in *L. branchialis*). However, Bastide-Guillaume *et al.* (1987) have doubts about the use of the antennary processes as a recognition mark. Their

Table 4—Means and S.D. of 7 body measurements (in mm) of *L. lusci* (source groups $LLTL_K$, $LLTL_H$ and LLCL) and *L. minuta* (source group LMPM). The first 6 measurements were adjusted for total length by covariance analysis

Host:	Sand goby	Dragonet	Bib	Bib
Group:	LMPM	LLCL	$LLTL_K$	$LLTL_H$
Length proboscis	1.22 (0.27)^b	1.40 (0.39)^b	1.77 (0.27)^a	1.37 (0.31)^b
Length neck	2.56 (0.48)^b	3.55 (0.92)^a	2.29 (0.13)^b	3.31 (0.59)^a
Length abdomen	2.93 (0.28)^b	3.27 (0.47)^a	3.41 (0.29)^a	3.14 (0.36)^{ab}
Width neck	0.51 (0.15)^c	0.74 (0.11)^a	0.61 (0.10)^b	0.67 (0.09)^{ab}
Width trunk	2.09 (0.33)^b	1.76 (0.28)^b	2.49 (0.34)^a	2.46 (0.27)^a
Width abdomen	1.12 (0.23)^b	0.95 (0.17)^b	1.48 (0.17)^a	1.33 (0.21)^a
Total length	9.90 (1.20)^b	14.7 (2.50)^a	16.46 (1.80)^a	15.86 (0.20)^a

Means with the same superscript in the same row are not significantly different (Tukey test for unequal sample sizes).

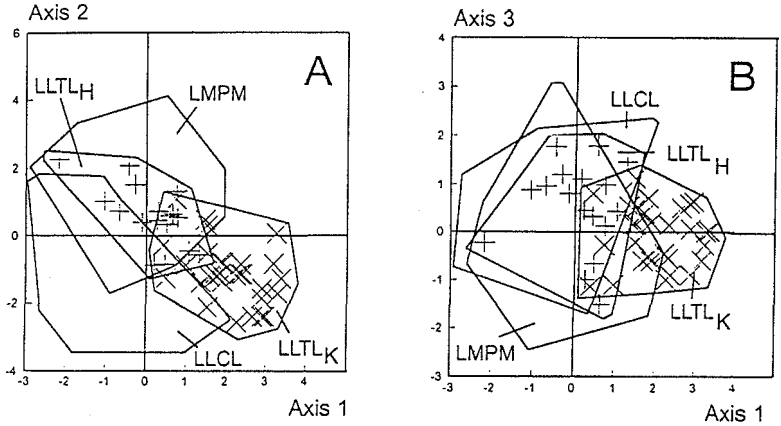

Fig. 7. Scatterplots derived from a canonical discriminant analysis on 4 *L. lusci* source groups after adjustment of body measurements for size-effects by Burnaby's method, for 4 source groups ($LLTL_K$, $LLTL_H$, LLCL, LMPM); (a) the plane formed by axes 1 and 2, (b) the plane formed by axes 1 and 3. The individuals of all groups are outlined. For clarity, only the individual observations of groups $LLTL_K$ and $LLTL_H$ are shown.

hypothesis is that these secondary structures, which secure attachment of *L. lusci* to the gill arches, might not develop in parasites which are attached to other sites. Counter-arguments were obtained during the present study: all *L. lusci* attached to uncommon places on bib (host surface, posterior ridges, operacula) developed antennary processes (though of different sizes). One single *L. branchialis* which was attached to gill arch 4 did not possess these structures. Moreover, Tirard (1991) found that all parasites possessing antennary processes were genetically distinct from parasites without processes. In conclusion, the antennary processes can indeed be used for the identification of these parasite species. These structures are clearly distinct from apparently analogous structures in *Lernaeenicus*, a copepod parasite of sprat. According to Raibaut, Berrebi & Rousset (1986) these latter structures, the presence of which was originally used as a criterion for discrimination between the 2 species, are morphological adaptations to the site of attachment on the host.

Though it is well documented that the size of animals is greatly influenced by environmental factors, this phenomenon is less well documented for parasitic crustaceans. It may occur especially in host–copepod systems in which individual hosts offer limited resources or in which parasite size is influenced by spatial restrictions. The latter factor may be of importance in *Lernaeocera* spp.: the size of these parasites is probably constrained by the size of the gill chamber. Since there also exists a relation between parasite size and reproductive output, it is obvious that parasites may have some advantage in infecting larger hosts. The effect of host size on the total length of *Lernaeocera* spp. has never been investigated in depth. In a recent study on the morphometry of *Lernaeocera lusci* (cf. Eiras & Santos, 1990) the host size is not even mentioned. Scott (1900) described *Lernaeocera minuta*, solely on the grounds of its small size as compared to the other species of the genus. Kabata (1979) considers *L. minuta* tentatively as a valid species, though he expresses doubts about the reliability of parasite size as a diagnostic criterion. The present study shows that parasite size is significantly influenced by host size and therefore should not be used as a criterion for species definition.

Parasite length is probably also influenced by other factors than host size. For example, the size of mature parasites may be age-specific rather than host size-dependent. This hypothesis could not be tested for *L. lusci* on *Trisopterus luscus*, because the age of the host and the time when the parasites had invaded their host were not known. A second factor which could possibly influence parasite size is intra-specific competition. This phenomenon, which is particularly well known for intestinal parasites (Read, 1959; Holmes, 1961), has not been described for copepod ecto- and mesoparasites. Evans *et al.* (1983) failed to find evidence for a crowding effect within *Lernaeocera lusci* infrapopulations. However, parasite length or weight may not be the best parameters to evaluate the crowding effect. Determining the reproductive

output may be more suitable for measuring density-dependent effects (Keymer, 1982).

A conclusion from this study is that parasite size should be discarded as a species characteristic because of its high dependence on host size. Therefore, we do not accept the validity of *L. minuta* and consider it to be a junior synonym of *L. lusci*.

Based on Kabata's key and considering the host-dependence of parasite size, only 2 valid species are accepted: *Lernaeocera lusci* and *L. branchialis*. Hence the proposal for the following key to the species of *Lernaeocera*:

Antennary processes present . . . . . *L. lusci*
Antennary processes absent . . . . . *L. branchialis*

The parasites on whiting all belong to the species *Lernaeocera branchialis*, and the parasites on bib, dragonet and sand goby all belong to the species *Lernaeocera lusci*.

In the past, specific characters used to distinguish between *Lernaeocera* species included the shape of the antlers (Scott & Scott, 1913; Stekhoven, 1936), the width of the neck (Kabata, 1957), the flexure of the abdomen (Kabata, 1957), the shape of the trunk (Kabata, 1957) and the structure of the cephalothoracic appendages (Stekhoven, 1936). At present, there is general agreement that most of these characters cannot be applied for description of *Lernaeocera* species (Kabata, 1979; Eiras & Santos, 1990) but a thorough study of the factors which affect the morphology of *Lernaeocera* spp. has never been undertaken. So far morphological characters do not seem to exist which would justify the description of more than 2 species. However, the question whether other criteria exist which can be used to discriminate between *Lernaeocera lusci* and *L. branchialis* should be further addressed.

The exploratory analyses on the raw data matrix yielded 100% separation between *Lernaeocera lusci* and *L. branchialis*. At first glance, this separation appears to result from size differences between the 2 species. Indeed, the first axis reflects an increasing gradient in size (Fig. 5), with the largest individuals (LBMM) being plotted at the one extreme and the smallest individuals (LMPM) at the other extreme end along this axis. However, it has been argued that parasite length is not a reliable criterion to separate *Lernaeocera* species due to its high dependence on host size. Hence the necessity to eliminate size-effects from the data matrix without losing information on shape.

Size-correction of morphometric measurements has been a controversial issue for many years. In a series of papers Atchley, Gaskins & Anderson (1976), Atchley (1978) and Atchley & Anderson (1978) warned against the use of ratios in morphometric studies. Humphries *et al.* (1981) instead proposed the use of complex multivariate adjustments. Burnaby's method as modified by Rohlf & Bookstein (1987), which was applied in the present study, is probably the most efficient way to remove size-effects of data matrices. Secondly, though in most studies size is removed from the dataset, it also contains valuable information (Bookstein, 1989). Failure to recognise the allometric consequences interdependent with size increase may lead to unwarranted taxon distinctions between organisms (Gould, 1966). In the present study allometric growth may be responsible for shape differences between groups. However, it may be difficult to disentangle the effect of host species, allometric growth and sites of attachment on shape characteristics. This may only be possible when a series of individual parasites is collected from one site of attachment on one host species.

There is little overlap between *L. lusci* (*L. minuta*) collected from different host species (dragonet, sand goby, bib): 91% of this species was allocated to the correct *L. lusci* source group after adjustment for size-effects with Burnaby's method. Besides size differences, other factors also contribute to this within-species discrimination. For example, in the exploratory analysis on the raw data matrix there is separation between the "smaller" LMPM, and the "larger" LLCL and LLTL along the first axis, which is determined mostly by total parasite length. The length of the proboscis and the length of the neck may be influenced by the site of attachment and by the distance to the nearest suitable blood vessel. The length of the proboscis is smallest in *L. branchialis* and longest in *L. lusci*. Within this latter group it is significantly longer in subgroup $LLTL_K$ than in subgroup $LLTL_H$. The neck of LLCL is longer than in all other groups. On the other hand, the posterior trunks may be influenced by spatial constraints, resulting in distortions of the general shape. For example, body width (width of abdomen and width of trunk) contributes significantly to the within-species discrimination of *L. lusci* (Table 3) and appears to be significantly larger in LLTL than in LLCL (Table 2).

In conclusion, the different biological forms encountered are shaped not only by the genetical differences between individuals, but also by the host species and the site of attachment. According to the International code of zoological nomenclature (1985) [Art. 1(b)(5)] a scientific name proposed expressly as the name of a "variety" or "form" after 1960 has the infrasubspecific rank and is excluded from zoological nomenclature. This rule justifies denotation of the 3 forms of *L. lusci* as *L. lusci* f. *lyra*, *L. lusci* f. *minuta*

and *L. lusci* f. *lusci*. Henceforth the biological forms of *L. lusci* will be called as such.

More objective methods, such as enzyme electrophoresis may have greater potential to determine the validity of species within this genus. Tirard (1991) found evidence for the absence of gene flow between the 2 species. She found that *L. branchialis* is monomorph for the loci glucose phosphate isomerase (GPI) and mannose phosphate isomerase (MPI), whereas *L. lusci* is polymorph for these loci. Absence of gene flow may be an adequate criterion for defining subpopulations as distinct species. Allozyme electrophoresis, when supported by morphological, life history or biogeographical data, may provide evidence for the existence of 2 or more species (Goater, 1990; Rannala, 1990).

Acknowledgements—We are grateful to T. Backeljau for giving advice about morphometry. Z. Kabata, R. Huys and A. Boxshall criticised earlier versions of the manuscript. The use of a scanning microscope was made possible by K. Wouters (K.B.I.N., Belgium). This study was supported by FKFO project 2.0086.88. P.A.V.D. was supported by the National Science Foundation of Belgium (NFWO).

REFERENCES

Atchley W. R., Gaskins C. & Anderson D. 1976. Statistical properties of ratios. I. Empirical results. *Systematic Zoology* 25: 137–148.

Atchley W. R. 1978. Ratios, regression intercepts and the scaling of data. *Systematic Zoology* 27: 78–83.

Atchley W. R. & Anderson D. 1978. Ratios and the statistical analysis of biological data. *Systematic Zoology* 27: 71–78.

Bastide-Guillaume C., Douëllou L., Romestand B. & Trilles J. P. 1987. Etude comparée des deux *Lernaeocera* hébergés respectivement par: *Merluccius merluccius* et *Trisopterus minutus capelanus* dans le golfe du Lion (Sète, France). *Revue des Travaux de l'Institut de Pêches maritimes* 49: 143–154.

Bookstein F. L. 1989. "Size and shape": a comment on semantics. *Systematic Zoology* 38: 173–180.

Burnaby T. P. 1966. Growth-invariant discriminant functions and generalized distances. *Biometrics* 22: 96–110.

De Meeus T., Renaud F. & Gabrion C. 1990. A model for studying isolation mechanisms in parasite populations: the genus *Lepeophtheirus* (Copepoda, Caligidae). *Journal of Experimental Zoology* 254: 207–214.

Eiras J. C. & Santos P. J. T. 1990. The morphology and morphometry of *Lernaeocera lusci* Bassett-Smith, 1893 (Copepoda, Pennellidae). *Publications of the Institute of Zoology "Dr. Augusto Nobre"* 219, 10 pp.

Evans N. A., Whitfield P. J., Bamber R. N. & Espin P. M. 1983. *Lernaeocera lusci* (Copepoda: Pennellidae) on bib (*Trisopterus luscus*) from Southampton Water. *Parasitology* 86: 161–173.

Goater T. M. 1990. Helminth parasites indicate predator-prey relationships in desmognathine salamanders. *Herpetological Reviews* 21: 32–33.

Gould S. J. 1966. Allometry and size in ontogeny and phylogeny. *Biological Reviews* 41: 587–640.

Holmes J. C. 1961. Effects of recurrent infections on *Hymenolepis diminuta* (Cestoda) and *Monoliformes dubius* (Acanthocephala) 3. Effects in hamsters. *Journal of Parasitology* 48: 97–100.

Humphries J. M., Bookstein F. L., Chernoff B., Smith G. R., Elder R. L. & Poss S. G. 1981. Multivariate discrimination by shape in relation to size. *Systematic Zoology* 30: 291–308.

Kabata Z. 1957. *Lernaeocera obtusa* n.sp., a hitherto undescribed parasite of the haddock (*Gadus aeglefinus* L.). *Journal of the Marine Biological Association of the U.K.* 36: 569–592.

Kabata Z. 1979. *Parasitic Copepoda of British Fishes*. The Ray Society, London.

Keymer A. 1982. Density-dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology* 84: 573–587.

Raibaut A., Berrebi P. & Rousset V. 1986. Utilisation de la génétique enzymatique pour comparer les copépodes parasites du genre *Lernaeenicus* (Pennellidae), selon la zone d'implantation dans l'hôte *Sardina pilchardus* (Walbaum, 1792). *Comptes rendues de l'Académie de Sciences* (série III) 303: 31–36.

Rannala B. H. 1990. Genetic distance and parasite taxonomy. *Journal of Parasitology* 76: 929–930.

Read C. P. 1959. The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. *Experimental Parasitology* 8: 365–382.

Rohlf F. J. & Bookstein F. L. 1987. A comment on shearing as a method for "size correction". *Systematic Zoology* 36: 356–367.

Scott T. 1900. Notes on some crustacean parasites of fishes. *Report of the Fishery Board of Scotland* 19: 144–188.

Scott T. & Scott A. 1913. *The British Parasitic Copepoda*. Vols I and II. The Ray Society, London.

Slinn J. D. 1970. An infestation of adult *Lernaeocera* (Copepoda) on wild sole, *Solea solea*, kept under hatchery conditions. *Journal of the Marine Biological Association of the U.K.* 50: 787–800.

Stekhoven J. H. 1936. Beobachtungen zur Morphologie und Physiologie der *Lernaeocera branchialis* und *L. lusci*. *Zeitung für Parasitenkunde* 8: 659–696.

Tirard C. 1991. Biodiversité et biogéographie évolutive dans les systèmes hôtes–parasites: le modèle Gadiformes (Téléostéens)—Copépodes et Monogènes. Thèse de doctorat, Université de Montpellier, France.

Van Damme P. A. & Hamerlynck O. 1992. The infection dynamics and dispersion pattern of *Lernaeocera branchialis* on 0+ whiting (*Merlangius merlangus* L.) in the Oosterschelde (SW Netherlands). *Journal of Fish Biology* 41: 265–275.

Van Damme P. A., Maertens D., Arrumm A., Hamerlynck O. & Ollevier F. 1993. The role of *Callionymus lyra* and *C. reticulatus* in the life cycle of *Lernaeocera lusci* (Bassett-Smith). *Journal of Fish Biology* 42: 395–401.

Van Damme P. A. & Ollevier F. 1994. The infection dynamics of *Lernaeocera lusci* (Bassett-Smith) on the sand goby (*Pomatoschistus minutus* Pallas) in the Oosterschelde. *Diseases of Aquatic Organisms* 19: 83–87.

Zeddam J. L., Berrebi P., Renaud F., Raibaut A. & Gabrion C. 1988. Characterization of two species of *Lepeophtheirus* (Copepoda, Caligidae) from flatfishes. Description of *Lepeophtheirus europaensis* sp. nov. *Parasitology* 96: 129–144.

HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 50, 177–190 (1996)

The population dynamics of *Lernaeocera lusci* and *L. branchialis* on intermediate hosts

P. A. Van Damme & F. Ollevier

Laboratory of Ecology, Zoological Institute; Naamsestraat 59, B-3000 Leuven, Belgium

ABSTRACT: The metapopulation dynamics of *Lernaeocera lusci* and *L. branchialis* on sole *Solea solea* and flounder *Pleuronectes flesus* were studied in the Dutch coastal area. Both fish species harboured large numbers of parasites when they arrived in the coastal area in the spring. Between April and June all parasites detached from the intermediate hosts and infected the definitive hosts (0+ whiting *Merlangius merlangus* for *L. branchialis*, and possibly sand goby *Pomatoschistus minutus* for *L. lusci*). Thereafter, flounder remained almost parasite-free until autumn. This suggests that *L. branchialis* has only 1 generation per year. However, soles were infested again with *L. lusci* (in June and July), which detached to infest 0+ bib *Trisopterus luscus*, the typical definitive host for this parasite species. Thus, it appears that *L. lusci* has 2 generations per year. The flounder length and the infection intensity of *L. branchialis* were not correlated throughout the study period. Significant positive correlations were found between the sole length and infection intensity of *L. lusci* in late spring, but not in the summer or autumn. Throughout the year, both *L. lusci* and *L. branchialis* were aggregated within their intermediate host populations (variance ⩾⩾ abundance).

INTRODUCTION

The life cycles of both *Lernaeocera branchialis* and *L. lusci* comprise two nauplius stages, a free-living copepodite stage and four chalimus stages on the intermediate host. After mating, the adult female leaves the intermediate host and infects the definitive host, usually a gadoid. In the southern North Sea, the typical definitive host species are bib (for *L. lusci*) and whiting (for *L. branchialis*). The population dynamics of both parasites on their definitive host species were recently studied in the Dutch coastal area (Van Damme & Hamerlynck, 1992; Van Damme et al., 1996).

Whereas the post-metamorphosis females of *L. branchialis* and *L. lusci* have received particular attention from fish parasitologists because of their size, their prominent position in the gill chamber of their definitive hosts and their pathogenicity, there is no comprehensive information available on the population dynamics of the parasitic stages on the intermediate hosts. Slinn (1970) found that the intermediate host of *L. lusci* is the sole *Solea solea*. The intermediate host of *L. branchialis* in the southern North Sea is the flounder *Pleuronectes flesus* (Kabata, 1979). Some quantitative data were provided by Stekhoven (1936), Sproston & Hartley (1941), Kabata (1958), Slinn (1970), Van den Broek (1979) and Whitfield et al. (1988). Anstensrud (1989, 1990a, 1990b, 1992) presented detailed accounts of the reproductive behaviour of *L. branchialis*; however, a thorough investigation of all population processes has never been undertaken. This study presents