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QUANTIFICATION OF MICROSCOPIC FEATHER CHARACTERS USED IN THE IDENTIFICATION OF NORTH AMERICAN PLOVERS[1]

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Abstract. Variation in microscopic characters of plumulaceous barbs of six species of *Charadrius* was studied to quantify characters and test differences among taxa. *Pluvialis squatarola* was used for intergeneric comparisons. Intraspecific variation in feathers from the sternopectoral tract of *C. vociferus* was examined to define character parameters and to determine within-vane variation and vane symmetry. A significant difference was observed among the barbs of four regions within each vane of the same feather, but barbs from opposing vanes were not significantly different from one another. Interspecific variation then was studied using discriminant function analysis. *Pluvialis* separated from all species of *Charadrius*. Two subgroups were apparent within *Charadrius*: *semipalmatus, vociferus* and *montanus* separated from *wilsonia, alexandrinus* and *melodus*.

Key words: plumulaceous, feathers, microcharacters, variation, Charadrius.

INTRODUCTION

The foundation of feather identification is in the work of Chandler (1916), who studied the systematic implications of feather microstructures to aid in understanding evolutionary relationships of birds. Since Chandler's time, identification of feathers and feather fragments has been applied to a variety of disciplines: paleontology (Steadman 1988, Humphrey et al. 1993, Laybourne et al. 1994), archeology (Hargrave 1965, Messinger 1965), feeding habits and prey remains (Day 1966, Gilbert and Nancekivell 1982, Griffin 1982, Ward and Laybourne, 1985), forensics (Davies 1970, Deedrick and Mullery 1981), food contamination (Olsen 1981), law enforcement (Laybourne, pers. comm.), and in bird-aircraft collisions or 'bird-strikes' (Manville 1963, Laybourne 1974, Brom 1991, Satheesen 1992).

Identification of a species from feathers is case specific, often depending on such factors as the amount and type of feathers and circumstantial evidence (e.g., locality, date, habitat) pertaining to the unknown sample. Traditional feather identification involves matching macroscopic characters (color pattern, shape, texture, size) to specimens in a reference collection of museum study skins and/or comparing the microscopic characters (nodal pigmentation and morphology) of the plumulaceous (downy) barbs with a microslide reference collection of known taxa using a comparison light microscope. The possible utility of micro-identification techniques in systematic research has led to questions concerning intraspecific variation among barbs of the same feather (Chandler 1916, Robertson et al. 1984) and among feather tracts of individuals (Day 1966, Reaney et al. 1978, Gilroy 1987). Therefore, a systematic approach to document interspecific variation is required. In this study I have attempted to provide a quantitative procedure for evaluating the two basic assumptions underlying feather identifications: first, relative high variability of feather microstructures within a species does not preclude discrimination among taxa if barbs from the same feather region and same area of the body are studied; and second, there are consistent differences between species that can be used as criteria upon which to base species identification. Therefore, this study examined both intra- and interspecific variation.

FEATHER STRUCTURE

Contour feathers consist of a rachis with vanes on either side and, in most birds, an afterfeather (Fig. 1). Most vanes are composed of two types of feather structures: stiff, pennaceous barbs

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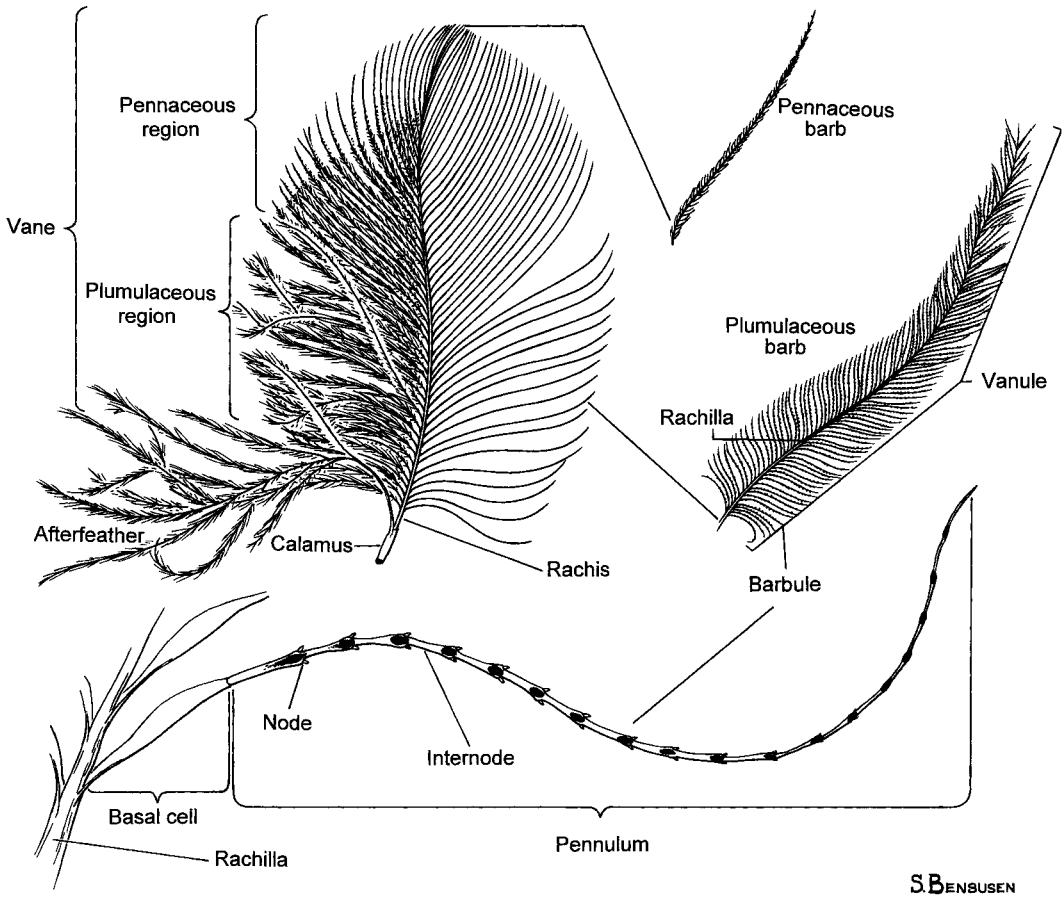


FIGURE 1. Topography of a contour feather.

that interlock and form the surface of a feather, and plumulaceous or downy barbs that have a fluffy appearance and are located at the base of the feather. Plumulaceous downy barbs are not to be confused with true down, which is a different type of feather. Barbs consist of a rachilla with vanules on either side which in turn are made up of barbules. Barbules, branching from barbs, are the smallest division of the feather and consist of a basal cell and a pennulum. It is the variation in microcharacters of nodal morphology, internodal length and width, basal cell length, pigmentation patterns, and pennulum (barbule) length that aid in the identification of groups of birds.

The subpennaceous region at the base of the plumulaceous barb (Fig. 2) has structural similarities to pennaceous barbs. Macroscopically, these basal barb areas give a distinct pennaceous texture near the rachis in some groups of birds (typical of Galliformes). Lucas and Stettenheim (1972) describe the barbules that make up this area as curled-base barbules, but the cumulative area of these barbules along the rachilla has not been given a descriptive term. Since this character proved significant in the interspecific comparisons it is here designated as the subpennaceous region of the barb (Figs. 2 and 4) because this region subtends the more distal pennaceous vanes. Barbule microstructures of this region also are important in determining vanule orientation of the barb; distal vanules (directed away from the body) have hooklet structures that are similar to those observed on distal pennaceous barbs.

METHODS

A high rate of birdstrike identifications in the genus *Charadrius* has provided opportunity for background knowledge of qualitative micro-

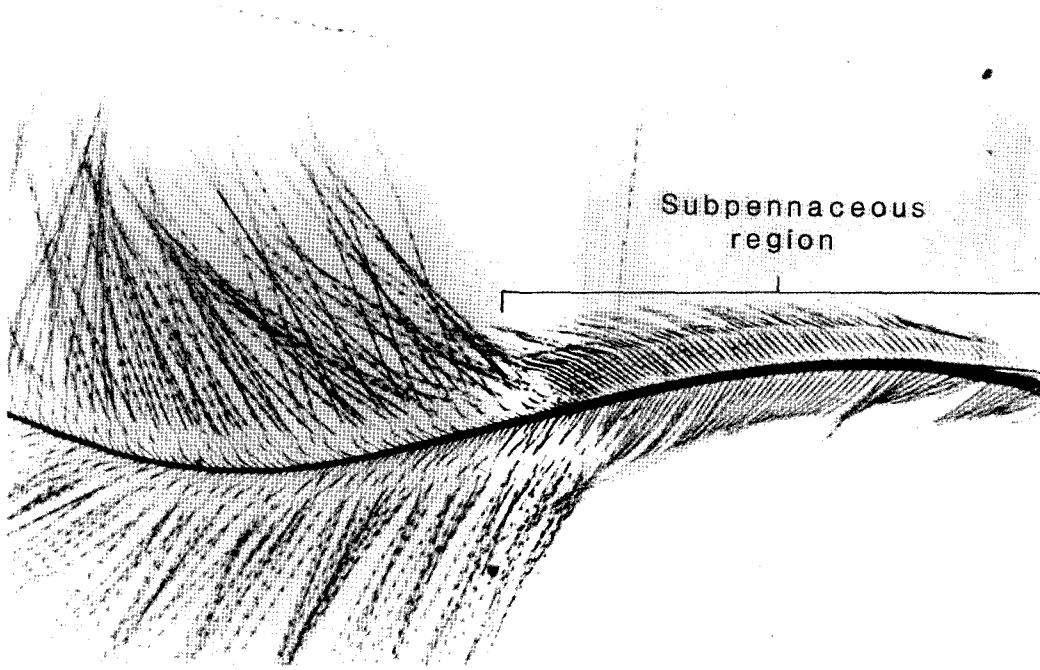


FIGURE 2. Subpennaceous region of *Charadrius melodus* (Intermediate region, 40×).

structural variations in the downy barbs of the species within this genus. *Pluvialis squatarola* (Black-bellied Plover) was used for intergeneric comparison.

Because basic knowledge of the variation in microstructures of the plumulaceous feather is minimal, quantification was undertaken at two levels. First, the amount of variation within a single type of feather (breast) was determined. After basic questions of vane symmetry, microcharacter parameters, and within-vane variation were determined, interspecific variation of closely related species was examined.

INTRASPECIFIC VARIATION

Charadrius vociferus was studied for individual variation. Because Chandler (1916), Day (1966) and Robertson et al. (1984) have cited differences in microscopic structures between adults and fledglings, and Messinger (1965) warned of possible differences in sex and age classes of other species, a sample of 10 adult males (museum specimens) was selected to determine intraspecific variation in vane symmetry and within-vane variation, and to define quantifiable characters. Contour feathers were removed from the sternopectoral tract (upper left breast) of each specimen; the afterfeathers were removed to avoid contamination of the feather type. Feathers were prepared according to the methods described in Laybourne and Dove (1994) and Sabo and Laybourne (1994). Light microscopy measurements were made using a compound light microscope with the ocular micrometer calibrated at 50x (low), 100x (mid) and 430x (high) power. Character measurements were examined by analysis of variance (ANOVA) and Student's *t*-tests using SYSTAT for Windows (SYSTAT, Inc. 1992).

The plumulaceous area of the feather was divided into eight regions, four on each vane (umbilical, basal, intermediate, and distal: A–D right vane; E–H left vane) to allow for study of the entire downy region of the feather (Fig. 3). Figure 4 illustrates character measurements for intra- and interspecific analysis and Table 1 defines the microcharacters measured initially.

INTERSPECIFIC VARIATION

Twenty adult museum specimens (ten males, ten females) from each of the following species were used for interspecific comparisons: *Charadrius semipalmatus* (Semipalmated Plover), *C. alexandrinus* (Snowy Plover), *C.*

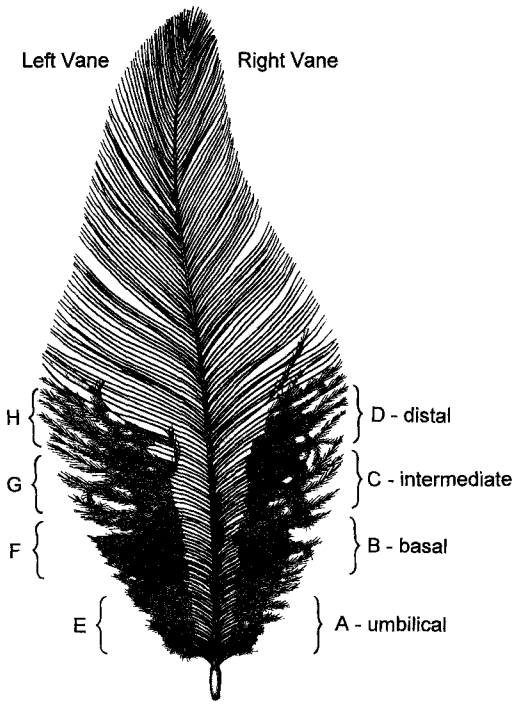


FIGURE 3. Plumulaceous regions of a contour feather.

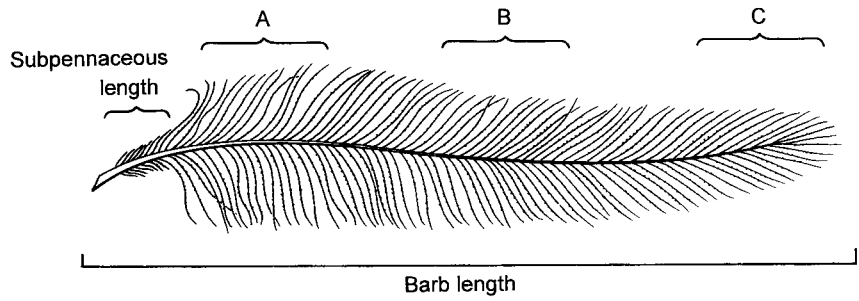
melodus (Piping Plover), *C. wilsonia* (Wilson's Plover), *C. montanus* (Mountain Plover), and *C. vociferus* (Killdeer). In this study, seasonal or geographical plumage variation in color, texture, or micromorphology did not affect the microstructures of downy barbules, and the down color of all species was similar. Therefore, specimens were selected without regard to locality, habitat type, and season. Characters were analyzed with a full factorial multivariate analysis of variance (MANOVA), and discriminant function analysis via SYSTAT (SYSTAT, Inc. 1992).

Because of the large sample size required for interspecific study, and to minimize damage to museum specimens from destructive sampling, barbs instead of whole feathers were sampled from the intermediate region, right vane of the upper left breast of each specimen. These barbs were mounted, two or three individuals to a slide, using round, 20 mm diameter, coverslips.

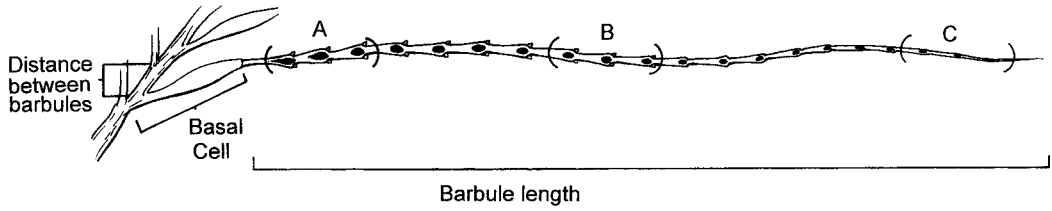
The following characters were retained from the intraspecific study for interspecific comparisons among *Charadrius*: barb length, subpennaceous length, barbule length, basal cell length, nodes per barbule (measurement of three separate barbules; basal, mid- and distal barbules on one barb, Fig. 4), and pigmented nodes

TABLE 1. Definitions of measurements of potentially useful characters for intraspecific study. Measurements made at high power (430×), mid-section of the barb unless otherwise noted.

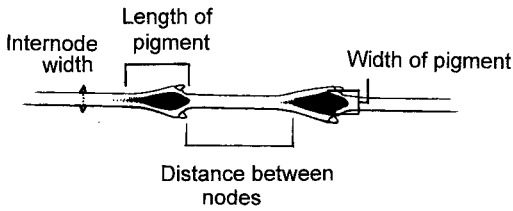
Character	Measurement
Barb length	50× Measured from point where barb is attached to rachis to the tip of barb.
Subpennaceous length	Measured from point where barb is attached to rachis to the point where normal downy barbules occur.
Barbule length	From basal cell division to tip of barbule. Also known as pennulum length.
Basal cell length	Measured from the attachment point on the rachilla to the first cell division.
Distance between nodes proximal/distal	Measurements taken at two points: on first or second proximal node from junction of one cell to next and on the next to last distal node.
Length of pigment proximal/distal	Length of pigmentation along axis of barbule on first or second proximal node and most distal nodes.
Width of pigment proximal/distal	Width of pigment at nodes, proximal and distal points.
Internode width	Pennulum width between node.
Spine length	Length of spine at proximal and distal nodes.
Distance between barbules	Measured at midsection of barb, distance from base of one barbule to the next distal barbule.
Pigmented part of pennulum	Portion of pennulum that had pigmented nodes.
Nodes per barbule	Total number of nodes counted on basal (A), mid (B), and distal (C) barbules (see Fig. 4, Barb).
Pigmented nodes per barbule	Same barbules as previous measurement except only counted pigmented nodes on each of the three barbules.



BARB - Barb length, Subpennaceous length. Barb sections: Basal (A), Mid-(B), and Distal (C).



BARBULE - Barbule length, Basal cell length, Distance between barbules. Barbule sections: Proximal (A), Mid-(B), and Distal (C).



NODE - Distance between nodes, Internode width, Length of pigment, Width of pigment.

FIGURE 4. Measurements taken on plumulaceous barbs, barbules and nodes.

per barbule of the same three barbules as the previous measurement. Measurements were concentrated on one vane, in one region within a vane to maximize consistency of characters among species.

RESULTS

INTRASPECIFIC VARIATION

Some of the characters were eliminated from further study during the intraspecific examination: width of pigment was eliminated due to poor resolution of the microscope. Length of pigment was disqualified due to difficulty in determining pigment boundaries; some of the proximal pigment extended from one node through the next and was not present on some distal nodes. Mechanical problems of resolution also prevented accurate measurements of internode width and spine length. Distance between barbules was impossible to measure accurately due to cross-over and flattening of barbules during slide preparation. The pigmented part of pennulum was considered to be a repetitive measurement of pigmented nodes per barbule, both of which were designed to determine

amount of pigment on barbules. The distance between proximal nodes character was eliminated due to lack of observed qualitative differences among species in a preliminary examination of all taxa. Distance between distal nodes was eliminated due to measurement difficulties in some species.

Vane symmetry. Symmetry of vanes (right and left) was tested by conducting two-tailed Student's *t*-tests on each character measured on barbs from opposing vanes (e.g., A=E, B=F, etc., Fig. 3). The alpha level = 0.01 was chosen for this test in order to provide a stringent test criterion for vane symmetry. Because all *t*-tests resulted in nonsignificant vane differences at this level, analysis was restricted to one vane for interspecific comparisons.

Although vanule (right and left sides of barb) asymmetry has been observed in some groups of birds, e.g., Trochilidae, Rallidae, and Columbidae (Gilroy 1987, Brom 1991, Laybourne, pers. comm., Dove, pers. observ.), it was not apparent during qualitative surveys of Charadriidae. In groups that exhibit vanule asymmetry, both vanes should be compared across taxa.

Within-vane variation. Differences within each vane were determined by one-way analysis of variance (ANOVA) on barbs within the right and left vanes. A significant difference ($P < 0.05$) was observed among the barbs of each vane for the subset of characters. An increase in barb length, barbule length, subpennaceous length, basal cell length, nodes per barbule (A and B), and pigmented nodes per barbule (A and B), was noted from the umbilical to the distal portion of the plumulaceous feather.

Significant variation within the vane warranted careful selection of a specific barb region for interspecific comparison. Since measurement values increased distally on the plumulaceous feather, the intermediate right vane region C (Fig. 3) was chosen because it represents an average position on the downy portion of the feather. This region also is easily accessible without having to remove the entire feather from the specimen.

INTERSPECIFIC VARIATION

Qualitative analysis. A qualitative survey of the microscopic characters of each species revealed visible differences in nodal morphology and pigmentation, basal cell pigmentation, and length and intensity of pigmentation of subpennaceous regions.

Members of the Charadriidae typically have medium-length barbs and barbules compared to those, for example, of passerines which are generally short and densely packed with pigmented nodes, or to Falconiformes, which typically have very long barbules and widely spaced unpigmented nodes (with the exception of Falconidae which have pigmented nodes). Most species of Charadriidae have distinct diamond-shaped pigmentation surrounded by a transparent area, and expanded basal nodes. Although some barbs and barbules are unpigmented, pigment is usually present to some degree in most barbs of all species. Spines are visible at most nodes even if pigmentation is lacking. When present, pigment is often heavily stippled internodally (posterior to the node) but does not extend to the tip of the barb or barbule in every species. Distal nodes are sometimes pigmented just below the node. Although Brom's (1991) study of European *Charadrius* describes the genus as usually having pigmented nodes only at the basal part of the most basal barbules, I found pigment at mid- and distal nodes on mid-section barbules of North American species (Table 2). The distance between the nodes appears to become greater distally as the internode tapers toward the tip of the barbule. With the exception of the first node, which is sometimes reduced in size, basal nodes are larger than distal nodes. Most species have some degree of spotted pigmentation within the basal cell. Subpennaceous regions are present on all species examined and have varying amounts of pigmentation. Distal vanules of the subpennaceous region are typically more heavily pigmented than the proximal.

The most distinct and consistently observed characters of plumulaceous feathers are compared and summarized by species as follows:

Charadrius semipalmatus.—The subpennaceous region of this species is heavily pigmented, medium-dark in coloration and medium-long compared to the other species in this group. Both the barbs and barbules are pigmented throughout. The pigment shape is more elongate and narrow when compared to *C. vociferus,* which has similar microscopic characteristics. Distal nodes of barbules are barely swollen and distal pigmentation is stippled internodally.

Charadrius alexandrinus.—The subpennaceous region of the feathers of this species varies from being very lightly to totally unpigmented. This region is small and not very apparent on most barbs. Barbules are relatively short. Barb pigmentation varies from almost no pigment to approximately two-thirds pigmented. Barbules that have pigment are almost never pigmented to the tip, and the first node is often unpigmented. Spines are visible at all nodes. Mid- and distal nodes are more swollen than in other unpigmented species in this group.

Charadrius melodus.—The short subpennaceous region is lightly pigmented, but more heavily than in *C. alexandrinus*. Barbs and barbules are short. Many barbs are unpigmented but, when observed, pigmented barbules extend to about half the length of the barb. When present, nodal pigmentation is more elongated than in *C. alexandrinus* and *C. wilsonia*. Spines are visible at all nodes along the barbule. Distal nodes are unpigmented and swollen.

Charadrius wilsonia.—The subpennaceous region is long with less intense pigmentation than in *C. vociferus*. The barb is pigmented to at least half its length (usually two-thirds) and the barbules are not pigmented to the tip. This species is more heavily pigmented than *C. alexandrinus* or *C. melodus*. Pigment is often stippled internodally instead of being well defined at the nodes. Spines are not visible on distal nodes.

Charadrius montanus.—A long subpennaceous area with medium pigmentation is typical of this species. The barbs and barbules are longer than in all *Charadrius* species studied and are pigmented throughout. However, the very tip of the barb is often devoid of pigment. Nodes are numerous and closer together and pigment is more concentrated at the node than in other species of this study. Distal nodes are slightly swollen and pigmented.

Charadrius vociferus.—The subpennaceous region is heavily pigmented and long. Barbs and barbules are heavily pigmented, with pigmentation extending to the tips of most barbs and barbules. Although this species most closely resembles *C. semipalmatus* and *C. montanus* microscopically, it has more pigmented nodes and a longer and darker subpennaceous region. Distal nodes have visible spines and pigment is concentrated at the nodes.

Pluvialis squatarola.—The subpennaceous region is more heavily pigmented and longer, and barbs are longer than *Charadrius*. Nodes are heavily pigmented and internodal pigmentation is often heavy. Barbs and barbules are pigmented almost entirely to the tips. The nodes are long and narrow compared to *Charadrius*. Spines are not visible on distal nodes and pigment is below the distal nodes.

Quantitative analysis. Descriptive statistics indicated that *C. montanus* had the most nodes per barbule, whereas *Pluvialus squatarola* had the longest barb length and the longest subpennaceous length. Measurements did not consistently increase with size of the species (Table 3). *Charadrius alexandrius, C. melodus* and *C. wilsonia* were not pigmented to the tip of the barb. All species except *C. alexandrinus* had subpennaceous regions long enough to measure.

Because the same barbule was measured for nodes per barbule and pigmented nodes per barbule, the percentage of pigmentation of the barbule could be calculated for barbules from proximal to distal points along the barb. *Charadrius semipalmatus, C. montanus, C. vociferus,* and *Pluvialis* have the highest percent (>50% on each barbule) of pigmented nodes per barbule, whereas *C. alexandrinus, C. wilsonia* and especially *C. melodus* had the least pigmentation (Table 2). In all species the highest percent of pigmentation was on the basal barbules (pigmented nodes/barbule A) and the lowest percent on distal barbules (pigmented nodes/barbule C). The coefficient of variation (CV) results in Table 3 provide the first insight into these microcharacters for studying variation within taxa. Coefficient of variation values were fairly consistent across taxa for each character; however, some species were highly variable in a few characters.

Significant sex differences were observed in barbule length (*C. semipalmatus*, $P = 0.01$, *C.*

TABLE 2. Percentage of pigmented nodes per barbule in species of *Charadrius* and *Pluvialis squatarola* measured on basal, mid-, and distal sections of barb.

Species	Basal	Mid-	Distal
C. semipalmatus	99	95	68
C. alexandrinus	53	24	0
C. melodus	49	0	0
C. wilsonia	78	48	0
C. montanus	100	95	69
C. vociferus	99	98	94
P. squatarola	87	82	64

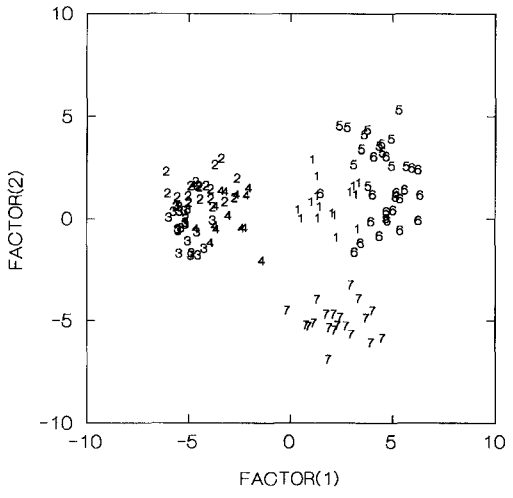


FIGURE 5. Discriminant function plot showing separation of species based on three characters:
Factor (1) = Pigmented nodes per barbule C, B.
Factor (2) = Subpennaceous length.
1 = *Charadrius semipalmatus*
2 = *Charadrius alexandrinus*
3 = *Charadrius melodus*
4 = *Charadrius wilsonia*
5 = *Charadrius montanus*
6 = *Charadrius vociferus*
7 = *Pluvialis squatarola*

wilsonia, $P = 0.03$), pigmented nodes per barbule A (*C. alexandrinus*, $P = 0.01$, *C. melodus*, $P < 0.01$ and *C. wilsonia*, $P = 0.04$), and pigmented nodes per barbule C (*C. montanus*, $P = 0.02$). To allow pooling of male and female measurements, the character pigmented nodes per barbule A was eliminated from further analysis because it showed sexual significance in three separate species.

Fully factorial multivariate analysis of variance (MANOVA) was used to examine changes in several properties simultaneously. MANOVA's using species as the factor resulted in highly significant differences among all species with both univariate F and multivariate test statistics ($F_{60,581} = 25.2$, $P < 0.001$). The first two discriminant factor variables (DFV) accounted for 98% of the variation among the groups. DFV 1, which explained 94% of the variation, loaded heavily on pigmented nodes per barbule C and pigmented nodes per barbule B, whereas DFV 2 (4% of variation) loaded heavily on subpennaceous length. A plot of DFV 1 and DFV 2 yielded good separation between three distinct groups (Fig. 5). DFV 1 separated *Charadrius* into two subgroups: *C. alexandrinus, C. melodus, C. wilsonia,* from *C. semipalmatus, C. montanus,* and *C. vociferus.* Better separation was evident between the two subgroups when *Pluvialis* was removed from the plot. Group membership versus predicted membership was greater than 90% in all but *C. vociferus* (86%), which was erroneously placed with *C. semipalmatus* twice and *C. montanus* once.

DISCUSSION

Previous researchers have been unable to provide a practical quantitative scheme for identifying feathers as to species or for quantifying interspecific differences among closely related species. Reaney et al. (1978) used scanning electron microscopy (SEM) to demonstrate that plumulaceous microstructures are valuable in identification of certain families of birds. However, that method is time-consuming, requires special microscopy facilities, and does not allow for detailed study of pigmentation patterns because SEM only provides a surface view. Robertson et al. (1984) quantified characters used in forensic feather identification, but their use of random areas within the plumulaceous feather and analysis of only two characters limited discrimination to ordinal levels at best. Shortcomings in those attempts to describe intraspecific variation prompted the unique approach of this study—the systematic comparison of nodes and barbules from a standardized, plumulaceous barb region of the same feather vane. This allowed examination of interspecific variation without having to deal with inconsistencies in feather regions or types. This procedure is not necessarily useful for identification because it may not be possible to determine from which part of the body the unknown sample came. However, the characters that have been used most often in qualitative microscopic identifications of this group of plovers (amount of pigmentation, subpennaceous region, barb length) provided the best separation of the species in this quantification procedure. It seems, therefore, that the best approach to feather identification is to use qualitative characters whenever possible rather than attempting to measure all possible microcharacters as was done initially in this study. When attempting to study microstructures of feathers, it is advisable to be-

gin by conducting a survey, such as the one described by Gilroy (1987), in order to become familiar with the different microstructures of various feather tracts.

This survey shows that significant differences exist in the plumulaceous feathers of North American plovers and that microcharacters of pigmented nodes per barbule C, pigmented nodes per barbule B, subpennaceous length, and barb length can be used to discriminate closely related species of this group. The characters selected for this study may or may not be appropriate in other groups of birds and certain groups may require a larger subset of characters than is presented here. For example, the barb and barbule length would be difficult to measure in groups of birds that have long flexuous barbules that extend beyond the field of microscopic view (e.g., Falconiformes or Galliformes). However, some of the characters that were eliminated in this study could prove useful with better light microscope resolution.

The possibility of omitting significant characters should have minimal effect if numerous characters are chosen. However, placing too much emphasis on qualitative characters is unwise. For example, the distance between distal nodes character seemed to be important in the qualitative survey but showed minimal quantifiable variation in this study. This study included a combination of meristic (basal cell length) and counted (nodes per barbule) characters. Because the latter loaded most heavily in discriminant function analysis, it would be advisable to begin with those characters and proceed with more tedious measurements if needed.

Additional characters that showed subjective interspecific variation were purposely avoided. Examples were color and intensity of nodal pigmentation, basal cell pigmentation, amount of interstippling of pigment through the internode, and round versus elongate nodal shapes. A combination of characters that allows examination of the whole barb and barbule provides the best approach to microanalysis of downy feathers.

Due to the small sample size of each sex ($n = 10$), rigorous testing of sexual differences in feather characters was not possible. A few characters showed significant sexual differences in some species but not in others, casting doubt on the reliability of pooling these characters. A

TABLE 3. Summary statistics of characters used for interspecific study of seven species of plover. Coefficient of variation in parenthesis, means ± SD.

Species	Character										
	Barb length	Subpennaceous length	Barbule length	Basal cell length	Nodes/ barbule A	Nodes/ barbule B	Nodes/ barbule C	Pigmented nodes/ barbule A	Pigmented nodes/barbule B	Pigmented nodes/barbule C	*n*
C. semipalmatus	4.89 ± 0.68 (0.14)	0.61 ± 0.16 (0.27)	0.92 ± 0.01 (0.10)	0.11 ± 0.01 (0.06)	19.8 ± 3.1 (0.16)	19.0 ± 2.6 (0.14)	16.9 ± 2.0 (0.12)	19.7 ± 3.0 (0.12)	18.0 ± 2.8 (0.16)	11.5 ± 3.6 (0.31)	20
C. alexandrinus	5.55 ± 0.71 (0.13)	0 (0)	0.88 ± 0.09 (0.10)	0.14 ± 0.18 (1.28)	19.7 ± 2.5 (0.13)	19.9 ± 1.7 (0.09)	15.8 ± 1.7 (0.11)	10.4 ± 7.7 (0.74)	4.8 ± 5.5 (1.16)	0 (0)	20
C. melodus	4.77 ± 0.60 (0.13)	0.52 ± 0.26 (0.50)	0.89 ± 0.08 (0.09)	0.09 ± 0.01 (0.14)	17.4 ± 2.6 (0.15)	18.4 ± 2.2 (0.12)	15.6 ± 1.8 (0.12)	8.5 ± 5.0 (0.59)	0 (0)	0 (0)	20
C. wilsonia	5.37 ± 0.50 (0.09)	0.63 ± 0.30 (0.48)	0.82 ± 0.01 (0.12)	0.10 ± 0.01 (0.10)	20.1 ± 1.3 (0.06)	18.3 ± 2.2 (0.12)	15.2 ± 2.3 (0.16)	15.8 ± 3.9 (0.25)	8.7 ± 6.1 (0.70)	0 (0)	20
C. montanus	6.77 ± 0.60 (0.09)	0.56 ± 0.43 (0.76)	1.16 ± 0.14 (0.12)	0.12 ± 0.01 (0.14)	28.2 ± 2.8 (0.10)	25.0 ± 2.7 (0.11)	20.3 ± 2.0 (0.10)	28.2 ± 2.8 (0.10)	23.7 ± 3.2 (0.13)	13.9 ± 3.2 (0.23)	20
C. vociferus	5.41 ± 0.73 (0.13)	0.87 ± 0.24 (0.27)	1.02 ± 0.10 (0.10)	0.10 ± 0.01 (0.13)	24.6 ± 2.8 (0.12)	23.1 ± 3.2 (0.14)	19.1 ± 2.8 (0.15)	24.5 ± 2.9 (0.12)	22.8 ± 3.3 (0.15)	17.9 ± 3.3 (0.19)	20
Pluvialis squatarola	7.73 ± 1.13 (0.15)	1.84 ± 0.43 (0.24)	1.10 ± 0.15 (0.13)	0.07 ± 0.02 (0.22)	19.1 ± 3.2 (0.17)	19.6 ± 2.3 (0.12)	15.7 ± 1.8 (0.11)	16.7 ± 3.2 (0.19)	16.0 ± 2.2 (0.14)	10.0 ± 2.9 (0.29)	20

larger sample size or examination of only one sex would avoid that problem.

Multivariate statistical analysis allowed a simultaneous examination of variation among all characters among groups. Because the species' identities were known, discriminant function analysis was used to determine maximum separation of groups in space. In this survey a large sample of individuals was necessary to insure an adequate biological representation of individual variation. Such sample sizes, especially of species that are rare or endangered, can only be obtained from museum collections of study skins.

Although the variability in some of these characters seems relatively high (Table 3) when compared with more standard skeletal measurements (Linsdale 1928, Larson 1930, Engles 1938), it did not preclude the use of these measurements for discriminating among the taxa (Fig. 5). When attempting identifications, the range of variation within a species is always a factor to consider, but experience has shown that consistent characters are present even when variation is high. In most cases of identification, sufficient feather material is present to examine both macro- and microscopically in conjunction with circumstantial evidence to reach a conclusive identification without detailed quantitative analysis. However, since the nature of discriminant analysis allows placing additional individuals into a data set, it is possible to take measurements of unknowns and predict the group to which they belong if (a) it is reasonably certain which part of the feather the unknown sample is from, (b) the same measurements are made on the unknown sample, and (c) the species identification has been narrowed to a minimum of possibilities for which a database of measurements has been created. Unfortunately, these requirements are rarely met with highly fragmented material.

This method may have systematic applications and could be used to complement other data sets. It is interesting to note that the interspecific patterns revealed here have some associations with the habitats characteristic of the species. Species within each of the *Charadrius* subgroups are split naturally by habitat type: *C. alexandrinus* and *C. wilsonia* prefer salt water habitats, whereas *C. montanus* and *C. vociferus* are fresh water species. The species that are found in intermediate aquatic habitats *C. semipalmatus* and *C. melodus* were split between the subgroups.

The quantification of plumulaceous microcharacters enhances the reliability of identifications and strongly suggests that microscopic feather characters can be used to discriminate closely related taxa. The method also corroborates the validity of microscopic identifications of unknown feather material.

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